(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

CORRECTED VERSION

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 October 2000 (12.10.2000)

PCT

(10) International Publication Number
WO 00/59532 A1

(51) International Patent Classification[7]: A61K 38/39

(21) International Application Number: PCT/US00/08678

(22) International Filing Date: 31 March 2000 (31.03.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/127,391 1 April 1999 (01.04.1999) US

(71) Applicant (*for all designated States except US*): **BIOSTRATUM, INC.** [US/US]; Suite 120, 2605 Meridian Parkway, Durham, NC 27713 (US).

(72) Inventors; and
(75) Inventors/Applicants (*for US only*): **BROOKS, Peter** [US/US]; 7054 Hawthorn Avenue, Hollywood, CA 90028 (US). **HUDSON, Billy** [US/US]; 15414 West 94th Street, Lenexa, KS 66219 (US).

(74) Agent: **HARPER, David, S.**; McDonnell, Boehnen, Hulbert [entity:amp] Berghoff, Suite 3200, 300 South Wacker Drive, Chicago, IL 60606 (US).

(81) Designated States (*national*): AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN. CR. CU, CZ, DE. DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP. KE, KG, KP. KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK. MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA. ZW.

(84) Designated States (*regional*): ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ. UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
with international search report

(48) Date of publication of this corrected version:
15 November 2001

(15) Information about Correction:
see PCT Gazette No. 46/2001 of 15 November 2001, Section II

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/59532 A1

(54) Title: THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AN TUMOUR GROWTH

(57) Abstract: The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interactions with the extracellular matrix, involving contacting the tumor, animal tissue, or endothelial cells with an amount effective to inhibit angiogenesis, tumor growth and metastasis, or endothelial cell interactions with the extracellular matrix of an antagonist of specific integrin receptors.

THE USE OF DOMAINS OF TYPE IV COLLAGEN T INHIBIT ANGIOGENESIS AN TUMOUR GROWTH

Cross Reference

This application claims priority to U.S. Provisional Application Serial No. 60/127,391 filed April 1, 1999, and is a continuation in part of U.S. Application Serial No. 09/277,665, filed March 26, 1999, which is a continuation in part of U.S. Application Serial No. 09/183,548 filed October 30, 1998, which is a continuation of 08/800,965 filed February 18, 1997, now U.S. Patent No. 5,856,184.

Field of the Invention

This invention relates to methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interactions with the extracellular matrix.

Background of the Invention

Angiogenesis, the process of formation of new blood vessels, plays an important role in physiological processes such as embryonic and postnatal development, as well as in wound repair. Formation of blood vessels can also be induced by pathological processes involving inflammation (e.g., diabetic retinopathy and arthritis) or neoplasia (e.g., cancer) (Folkman, 1985, Perspect, Biol. Med., 29, 10). Neovascularization is regulated by angiogenic growth factors secreted by tumor or normal cells as well as the composition of the extracellular matrix and by the activity of endothelial enzymes (Nicosia and Ottinetti, 1990, Lab. Invest., 63, 115).

During the initial stages of angiogenesis, endothelial cell sprouts appear through gaps in the basement membrane of pre-existing blood vessels (Nicosia and Ottinetti,

1990, *supra*; Schoefl, 1963, Virehous Arch, Pathol. Anat. 337, 97-141; Ausprunk and Folkman, 1977, Microvasc. Res. 14, 53-65; Paku and Paweletz, 1991, Lab. Invest. 63, 334-346). As new vessels form, their basement membrane undergoes complex structural and compositional changes that are believed to affect the angiogenic response (Nicosia, et. al., 1994, Exp. Biology, 164, 197-206). Early planar culture models have shown that basement membrane molecules modulate the attachment, migration, proliferation, and organizational behavior of endothelial cells (Nicosia, et. al., 1994, *supra*). More recent studies with three-dimensional aortic culture models that more closely simulate angiogenic conditions during wound healing in vivo suggest that the basement membrane is a dynamic regulator of angiogenesis, and its function varies according to its molecular components (Nicosia, 1994, *supra*).

A common feature of all solid tumor growth is the requirement for a blood supply. Therefore, numerous laboratories have focused on developing anti-angiogenic compounds based on growth factors and their receptors. While this approach has led to some success, the number of growth factors known to play a role an angiogenesis is large. Therefore, the possibility exists that growth factor antagonists may have only limited use in treating cancer since tumors and associated inflammatory cells likely produce a wide variety of factors that can induce angiogenesis.

In this regard, a strategy that targets a common feature of angiogenesis, such as endothelial cell adhesion to the extracellular matrix (ECM), might be expected to have a profound physiological impact on tumor growth in humans. This notion is supported by the fact that RGD-containing antagonists of the αvβ3 integrin ECM cell adhesion receptor can block angiogenesis. (U.S. Patent No. 5,766,591) Furthermore, the αvß3 integrin is expressed most prominently on cytokine -activated endothelial and smooth

muscle cells and has been shown to be required for angiogenesis. (Varner et al., Cell Adhesion and Communication 3:367-374 (1995); Brooks et al., Science 264:569-571 (1994)). Based on these findings, a potentially powerful new approach to anti-angiogenic therapy might be to specifically target critical regulatory domains within distinct ECM components.

The basement membrane (basal lamina) is a sheet-like extracellular matrix (ECM), which is a basic component of all tissues. The basal lamina provides for the compartmentalization of tissues, and acts as a filter for substances traveling between tissue compartments. Typically the basal lamina is found closely associated with an epithelium or endothelium in all tissues of an animal including blood vessels and capillaries. The basal lamina components are secreted by cells and then self assemble to form an intricate extra-cellular network. The formation of biologically active basal lamina is important to the development and differentiation of the associated cells.

Type IV collagen has been shown to be a major structural component of basement membranes. The protomeric form of type IV collagen is formed as a heterotrimer made up from a number of different subunit chains called $\alpha1$(IV) through $\alpha6$(IV). Up to now, six genetically distinct α-chains belonging to two classes with extensive homology have been identified, and their relative abundance has been demonstrated to be tissue specific. The type IV collagen heterotrimer is characterized by three distinct structural domains: the non-collagenous (NC1) domain at the carboxyl terminus; the triple helical collagenous domain in the middle region; and the 7S collagenous domain at the amino terminus. (Martin, et. al., 1988, Adv. Protein Chem. 39:1-50; Gunwar, et. al. 1991, J. Biol. Chem. 266:14088-14094).

The ability to express recombinant α(IV) NC1 domains provides the opportunity to study the effect of specific domains on many biological processes, such as angiogenesis, tumor metastasis, cell binding to basement membranes, and assembly of Type IV collagen molecules.

Summary of the Invention

The instant invention provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the extracellular matrix, each method comprising contacting the tumor, animal tissue, or endothelial cells with antagonists of specific integrin receptors.

Brief Description of the Drawings

Figure 1 illustrates the effects of NC1 (Hexamer) and 7S domains of Type IV collagen at a 50 μg/ml concentration on angiogenesis from mouse thoracic aorta organ cultures.

Figure 2 illustrates the effects of 7S domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μg/ml (control); 0.5 μg/ml; 5 μg/ml and 50 μg/ml.

Figure 3 illustrates the effects of NC1 (Hexamer) domain of Type IV collagen on angiogenesis from mouse thoracic aorta organ cultures. The domain concentrations employed in this experiment were 0 μg/ml (control); 5 μg/ml and 5 μg/ml and 50 μg/ml.

Figure 4 are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture. Control specimen (0 μg/ml of NC1 (Hexamer) and 7S domains)

exhibited growth of microvessels from the cultured tissue into the matrix (Figure 4A). In contrast, angiogenesis was inhibited in specimens cultured with 50 μg/ml of 7S domain (Figure 4B) and NC1 (Hexamer) domain (Figure 4C).

Figure 5 is a graphical representation of data demonstrating the in vivo effect of IV injection of recombinant (α1) type IV collagen monomer on angiogenesis using fibrin implants in rats.

Figure 6 is a graphical representation of data demonstrating that the recombinant (α1) and (α2) NC1 monomers inhibit the bFGF-induced increase in angiogenic index in vivo.

Figure 7 is a graphical representation of demonstrating the dose response effect of recombinant (α2) NC1 monomer on the bFGF-induced increase in total blood vessel branch points in vivo.

Figure 8 is a graphical representation of data demonstrating the dose response effect of recombinant (α2) NC1 monomer on the bFGF-induced increase in angiogenic index in vivo.

Figure 9 is a graphical representation of data demonstrating the dose response effect of recombinant (α2) NC1 monomer on the bFGF-induced increase in angiogenic index in vivo.

Figure 10 is a graphical representation of data demonstrating the effect of recombinant (α1) and (α2) NC1 monomers on mean CS-1 melanoma tumor weight in vivo.

Figure 11 is a graphical representation of data demonstrating the dose response effect of recombinant (α2) NC1 monomer on mean CS-1 melanoma tumor weight in vivo.

Figure 12 is a graphical representation of data demonstrating the effect of recombinant (α1), (α2), and (α4) NC1 monomers on mean HT1080 tumor weight in vivo.

Figure 13 is a graphical representation of data demonstrating the effect of recombinant (α1), (α2), (α3) and (α5) NC1 monomers on mean HEP-3 tumor weight in vivo.

Figure 14 is a graphical representation of data demonstrating human endothelial cell adhesion to immobilized NC1 α monomers.

Figure 15 is a graphical representation of data demonstrating the effect of soluble α1 and α2 NC1 monomers on human endothelial cell adhesion to pepsinized collagen type IV.

Figure 16 is a graphical representation of data demonstrating the effect of isolated recombinant NC1 monomers on human endothelial cell migration in vitro.

Figure 17 A-F provides the sequences of each type IV collagen α chain monomer.

Figure 18 is a graphical representation of data demonstrating the effect of monoclonal antibodies against various integrins on human endothelial cell adhesion to recombinant the (α2) NC1 domain.

Figure 19 is a graphical representation of data demonstrating human endothelial cell adhesion to the recombinant (α1) NC1 domain.

Description of the Preferred Embodiments

Within this application, unless otherwise stated, the techniques utilized may be found in any of several well-known references such as: *Molecular Cloning: A Laboratory Manual* (Sambrook, et al., 1989, Cold Spring Harbor Laboratory Press), *Gene Expression Technology* (Methods in Enzymology, Vol. 185, edited by D. Goeddel, 1991. Academic Press, San Diego, CA), "Guide to Protein Purification" in *Methods in Enzymology* (M.P. Deutshcer, ed., (1990) Academic Press, Inc.); *PCR Protocols: A Guide to Methods and Applications* (Innis, et al. 1990. Academic Press,

San Diego, CA), *Culture of Animal Cells: A Manual of Basic Technique, 2nd Ed.* (R.I. Freshney. 1987. Liss, Inc. New York, NY), and *Gene Transfer and Expression Protocols*, pp. 109-128, ed. E.J. Murray, The Humana Press Inc., Clifton, N.J.).

As used herein, the term Type IV collagen domain encompasses the group of molecules including the non-collagenous NC1 domain (Hexamer) and 7S collagenous domains, as well as NC1 α chain monomers.

The invention comprises methods for using Type IV collagen NC1 α-monomers (ie: $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha6$), which are defined to include such monomers isolated from any multicellular organism or produced via recombinant protein expression from a gene encoding such a monomer from any multicellular organism, and also to encompass various modifications, additions, and/or deletions to such monomers.

In one aspect, the present invention provides methods and kits for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha6$ NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha6$ NC1 chain monomers.

In another aspect, the present invention provides methods and kits for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit metastasis of a polypeptide composition comprising one or more

isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha6$ NC1 chain monomers.

In a further aspect, the present invention provides methods and kits for inhibiting endothelial cell interactions with the extracellular matrix in tissue comprising contacting the tumor or tissue with an amount effective to inhibit endothelial cell interactions with the extracellular matrix of a polypeptide composition comprising one or more isolated type IV collagen NC1 α chain monomers selected from the group consisting of $\alpha1$, $\alpha2$, $\alpha3$, and $\alpha6$ NC1 chain monomers.

The NC1-encoding domain of each of the six α chain cDNAs has been cloned into a vector for recombinant protein expression as previously described (Sado et al., Kidney Intl. 53:664-671 (1998), incorporated by reference herein in its entirety). The vectors are used to stably transfect human kidney 293 cells, which produce the recombinant protein. The DNA and deduced amino acid sequences of the recombinant type IV collagen alpha chain monomers produced as described are shown in Figure 17A-F. The first 17 amino acids correspond to a BM40 signal sequence (which is cleaved from the mature protein), to facilitate protein secretion. All the secreted proteins (ie: mature proteins) start with the sequence APLA followed by the affinity tag, DYKDDDDK at the amino terminus. This tag facilitates purification and identification of the material, and does not interfere with biological activity of the recombinant NC1 α chain monomers.

The type IV collagen NC1 α chain monomers can be produced by any method known in the art, including using recombinant DNA technology or biochemical peptide synthesis technology, or by isolating the NC1 domains from animal sources, such as from basement membrane sources such as bovine lens capsule and bovine kidney glomeruli.

(Peczon et al., Exp. Eye Res. 30:155-165 (1980); Langeveld et al., J. Biol. Chem. 263:10481-10488 (1988); Gunwar et al., J. Biol. Chem. 266:14088-14094 (1991))

In practicing the invention, the amount or dosage range of type IV collagen NC1 α chain monomers employed is one that effectively inhibits angiogenesis, tumor growth, tumor metastasis, and/or endothelial cell-extracellular matrix interactions. An inhibiting amount of NC1 α chain monomers that can be employed ranges generally between about 0.01 μg/kg body weight and about 10 mg/kg body weight, preferably ranging between about 0.05 μg/kg and about 5 mg/kg body weight.

The NC1 α chain monomers may be administered by any suitable route, including orally, parentally, by inhalation spray, rectally, or topically in dosage unit formulations containing conventional pharmaceutically acceptable carriers, adjuvants, and vehicles. The term parenteral as used herein includes, subcutaneous, intravenous, intraarterial, intramuscular, intrasternal, intratendinous, intraspinal, intracranial, intrathoracic, infusion techniques or intraperitoneally. In preferred embodiments, the NC1 α chain monomers are administered intravenously or subcutaneously.

The NC1 α chain monomers may be made up in a solid form (including granules, powders or suppositories) or in a liquid form (*e.g.*, solutions, suspensions, or emulsions). The NC1 α chain monomers of the invention may be applied in a variety of solutions. Suitable solutions for use in accordance with the invention are sterile, dissolve sufficient amounts of the NC1 α chain monomers, and are not harmful for the proposed application.

The NC1 α chain monomers may be subjected to conventional pharmaceutical operations such as sterilization and/or may contain conventional adjuvants, such as preservatives, stabilizers, wetting agents, emulsifiers, buffers etc.

For administration, the NC1 α chain monomers are ordinarily combined with one or more adjuvants appropriate for the indicated route of administration. The compounds may be admixed with lactose, sucrose, starch powder, cellulose esters of alkanoic acids, stearic acid, talc, magnesium stearate, magnesium oxide, sodium and calcium salts of phosphoric and sulphuric acids, acacia, gelatin, sodium alginate, polyvinylpyrrolidine, and/or polyvinyl alcohol, and tableted or encapsulated for conventional administration. Alternatively, the compounds of this invention may be dissolved in saline, water, polyethylene glycol, propylene glycol, carboxymethyl cellulose colloidal solutions, ethanol, corn oil, peanut oil, cottonseed oil, sesame oil, tragacanth gum, and/or various buffers. Other adjuvants and modes of administration are well known in the pharmaceutical art. The carrier or diluent may include time delay material, such as glyceryl monostearate or glyceryl distearate alone or with a wax, or other materials well known in the art.

The present invention may be better understood with reference to the accompanying examples that are intended for purposes of illustration only and should not be construed to limit the scope of the invention, as defined by the claims appended hereto.

Example 1 – In Vitro Effect on Angiogenesis

With modifications, the procedures of Nicosia and Ottinetti (1990), *supra*, and Nicosia, et. al. (1994), *supra*, were utilized for experiments designed to test the effect of Type IV collagen on angiogenesis under *in vitro* conditions. The model has been used to study the effects of growth factors and extracellular matrix molecules on the

angiogenic response and employs aortic rings cultures in three-dimensional collagen gels under serum-free conditions. These experiments are outlined below.

A. Methods

Experiments were performed with 1-3 month old Swiss Webster male mice. Following anesthesia, the thoracic aorta was excised under aseptic conditions and transferred to sterile MCDB 131 sterile growth medium (Clonetics, San Diego, CA) containing antibiotics. Fat was dissected away from the aorta and approximately six to eight 1 mm thoracic segments were obtained from each specimen. Segments were transferred to 48 well tissue culture plates. The wells of these plates were layered with 100 microliters of Matrigel (EHS basement membrane, Collaborative Biomedical Products, Bedford, MA) prior to transfer of the aortic segments. The Matrigel was diluted 1:1 with MCDB 131 growth medium prior to use. The segments were centered in the wells and an additional 100 microliters of Matrigel was then placed over the specimens. The aortic segments were therefore embedded in the basement membrane matrix. Each well then received 300 microliters of MCDB 131 growth medium. The plates were placed in an incubator maintained at 37° C with 5% CO_2. Specimens were observed daily over a 7 day period. Newly growing microvessels were counted using an inverted phase microscope at various times during the culture period, but data is expressed at 3 and 5 days of culture. To test for the effect of Type IV collagen on angiogenesis, domains at known concentrations were mixed with the Matrigel and with the MCDB 131 growth medium. Fresh MCDB 131 growth medium (plus and minus collagen domains) was changed every 3 days.

B. Results

After establishing the time course of angiogenesis under control conditions (Matrigel plus MCDB 131 growth medium), experiments were performed using various concentrations of Type IV collagen (isolated from bovine lens) NC1 (hexamer) and 7S domains. Data represents the analysis of at least 3 specimens per experimental condition. In the first experiment (**Figure 1**), analysis indicated that at a concentration of 50 μg/ml, NC1 domain and 7S domain significantly inhibited angiogenesis as monitored at 3 and 5 days of culture. In the second experiment, various concentrations of these domains were analyzed. As indicated in **Figure 3**, 7S domain at 50 μg/ml again significantly inhibited angiogenesis at 3 and 5 days. Inhibition was reduced at 5 and 0.5 μg/ml concentrations. As indicated in **Figure 2**, NC1 domain was less effective in blocking angiogenesis as compared to that observed in the first experiment (**Figure 1**), although it was still effective. In addition, as compared to the 7S domain, there was less of a correlation between concentration and inhibitory action.

Figure 4A-C are photographs of mouse thoracic aorta segments embedded in Matrigel (EHS basement membrane matrix, Collaborative Biomedical Products, Bedford, MA) at 5 days of culture in the presence or absence of 50 μg/ml of Type IV collagen domains. The control specimen (no domains) exhibited growth of microvessels from the cultured tissue into the matrix (**Figure 4A**). In contrast, angiogenesis inhibition was observed in tissues cultured in the presence of 50 μg/ml of 7S (**Figure 4B**) and NC1 (Hexamer) domain (**Figure 4C**).

Example 2. Subcutaneous fibrin implant angiogenesis

Recombinant human type IV collagen NC1 (α3) monomer (Sado et al., Kidney International 53:664-671 (1998)) was injected intravenously in Fisher 344 rats containing fibrin implants surgically placed subcutaneously, a modified version of the method described by Dvorak et al (Lab. Invest. 57(6):673-686 (1987)). The implants were then removed and directly analyzed using an inverted microscope. The analysis involved counting the number of blood vessels that had grown into the fibrin in the control and experimental group.

Briefly, 4 fibrin implants were surgically implanted subcutaneously into Fisher 344 rats (2 dorsal and 2 ventral sides). The average rat weight was approximately 125 grams.

Three rats (EXP) were given tail vein injections of either control (fibrin alone), 100 μl of 100 μg/ml of 7S domain of type IV collagen (approximately 0.80 mg/kg body weight), 100 μl of 100 μg/ml of type IV collagen hexamer (approximately 0.80 mg/kg body weight), or recombinant collagen type IV NC1 (α3) monomer at a concentration of 1.26 mg/ml in PBS (120 μg protein, or approximately 0.96 mg/kg body weight) and 3 rats (C) were given 100 μl tail vein injections of PBS. Injections of recombinant protein were given every other day for five doses. The injection schedule was as follows:

Day 1: (implant day) injection and remove blood sample (EXP and C)
Day 3: Injection (EXP and C)
Day 5: Injection and remove blood sample (EXP and C)
Day 7: Injection (EXP and C)
Day 9: Injection and remove blood sample (EXP and C)
Day 11: Remove and fix implants (save blood sample) (EXP and C)

The results of one experiment were as follows:

<u>2 week in vivo experiment</u>:

Control (fibrin alone)	about 66 BV

7S domain of type IV lens collagen (100 μg/ml)	None
Hexamer of type IV lens collagen (100 μg/ml)	None
Monomer (α3)	None

The results are shown as the mean number of blood vessels per implant. The results of this study demonstrate that isolated domains of type IV collagen, including the α3 monomer, can significantly inhibit capillary growth in the in vivo fibrin clot implant model. In subsequent experiments, the inhibitory effect was occasionally seen to attenuate with time, suggesting that higher dosages or more frequent injections might be even more effective.

A similar experiment was conducted using recombinant human type IV collagen NC1 (α1) monomer (100 μl of a 1 μg/μl solution; approximately 0.80 mg/kg body weight) and comparing the number of blood vessels that had grown into the fibrin at day 11 of treatment relative to the control group. Three rats per group were analyzed with each rat having 4 implants. These experiments demonstrated that administration of the α1 monomer significantly inhibited capillary growth in the in vivo fibrin clot implant model (Figure 5).

Example 3. Recombinant NC1 (α2) domain inhibits angiogenesis in vivo

We next tested the effects of systemic administration of soluble NC1 α-chain monomers in the chick embryo CAM angiogenesis assay.

Angiogenesis was induced in the CAMs of 10 day old chick embryos with bFGF as described (Brooks et al., Cell 92:391-400 (1998)). Twenty four hours later the embryos were systemically treated with various concentrations of recombinant NC1 α-chain monomers, in a total volume of 100 μl of sterile phosphate buffered saline (PBS). Two days later the embryos were sacrificed and the filter discs and CAM tissues

removed. Angiogenesis was quantitated by counting the number of angigogenic blood vessel branch points in the confined area of the filter disc. The Angiogenic Index is defined as the number of branch points from experimental treatment minus control treatment.

In initial experiments, recombinant α1 or α2 NC1 domains were injected at a concentration of 50 μg per embryo. At this concentration, the NC1 domains were shown to be highly toxic as demonstrated by greater than 90% embryo cell death. However, at lower doses they were well tolerated and showed potent anti-angiogenic activity. A total of 6 individual angiogenesis experiments were conducted with the NC1 domains. However, in two experiments, the bFGF induction was low, making it difficult to interpret the results. The NC1 α2 domain appeared to be more consistent and potent than the α1 NC1 domain at inhibiting angiogenesis. In fact, systemic administration of 30 μg of NC1 α2 consistently inhibited angiogenesis by greater than 90% (**Figures 6-9**), as measured by inhibition of the bFGF-induced increase in the angiogenic index and the mean number of blood vessel branch points. In contrast, NC1 α1 domain showed variable inhibitory activity (0%-50%) throughout the experiments.

<u>Example 4. Recombinant NC1 domain inhibits melanoma tumor growth in vivo:</u>

Since the growth of all solid tumors depends on angiogenesis to provide nutrients for its continued expansion, reagents that have the capacity to inhibit angiogenesis may significantly inhibit tumor growth. Therefore, we tested the effects of recombinant NC1 domains of type IV collagen for their effects on tumor growth in vivo.

To test the effects of NC1 domains on tumor growth in vivo, we utilized the chick embryo tumor growth assay. Briefly, single cell suspensions of 3 distinct tumor types were applied to the CAM of 10 day old chick embryos. The tumors included CS-1 Melanoma cells (5×10^6), HT1080 human fibrosarcoma cells (4×10^5) and Hep-3 human epidermoid carcinoma cells (2×10^5). The embryos were injected systemically with varying concentrations of NC1 α-chain monomers 24 hours later. The embryos were next allowed to incubate for a total of 7 days, at which time they were sacrificed. The resulting tumors were resected and wet weights determined. A total of 6 tumor growth assays were conducted with the 3 distinct tumor types. A single injection of 10 μg NC1 α2 domain inhibited CS1 melanoma tumor growth by approximately 70% relative to control **(Figure 10)**. In similar experiments, dose response curves were completed with CS-1 tumors. Systemic administration of NC1 α2 resulted in a dose-dependent inhibition of CS-1 melanoma tumor growth in vivo with a maximum inhibition following a single dose at 30 μg **(Figure 11)**. Systemic administration of NC1 α1 also inhibited CS-1 tumor growth but it was variable and in some experiments failed to inhibit tumor growth **(See Figure 10)**. In similar experiments, NC1 α2 inhibited HT1080 human fibrosarcoma tumor growth by approximately 50% after a single systemic injection of 30 μg, while NC1 α1 and α4 had no effect **(Figure 12)**. Finally, systemic administration of NC1 α2 (30.0 μg) and α3 inhibited Hep-3 human epidermoid carcinoma tumor growth by approximately 40% and 60% respectively, and α1 inhibited Hep-3 tumor growth by approximately 30%, while NC1 α5 domain failed to inhibit tumor growth **(Figure 13)**.

We conclude from these in vivo studies that tumor growth can be inhibited by isolated NC1 α-chain monomers. These molecules can thus be used alone, or to

complement the use of existing anti-tumor agents, in providing enhanced and more effective anti-tumor therapy.

Example 5. Immobilized NC1 domains support human endothelial cell adhesion

In order for new blood vessels to form, endothelial cells must have the capacity to adhere and migrate through the ECM. Moreover, this endothelial cell-ECM interaction may facilitate signal transduction events required for new blood vessel formation. Therefore, since type IV-collagen is an ECM protein which is known to support cell adhesion, we tested the ability of the NC1 domains to support endothelial cell attachment.

Microtiter plates were coated with 25 μg/ml of purified NC1 domains followed by incubation with 1% bovine serum albumin (BSA) to block non-specific interactions. Human endothelial cells (ECV304) were then allowed to attach to the immobilized NC1 domains for 1 hour. Non-adherent cells were removed by washing and attached cells were quantified by measuring the optical density (O.D.) of crystal violet eluted from attached cells. Data bars represent the mean +/- standard error of the O.D. from triplicate wells.

Immobilized NC1 $\alpha 2$, $\alpha 3$, and $\alpha 6$ domains supported endothelial cell adhesion while NC1 $\alpha 1$, $\alpha 4$, and $\alpha 5$ domains promoted little if any cell adhesion (**Figure 14**). Soluble NC1 $\alpha 1$ (a1) and $\alpha 2$ (a2) inhibited endothelial cell adhesion to pepsinized collagen type IV by approximately 50% (**Figure 15**).

Taken together, these findings demonstrate that isolated, recombinant NC1 domains from the $\alpha 1$, $\alpha 2$, $\alpha 3$, and $\alpha 6$ chains of collagen type IV·can mediate human endothelial cell adhesion and/or inhibit endothelial cell adhesion to ECM proteins in

vitro, and suggest that the potent anti-angiogenic and anti-tumor activity of the isolated NC1 domains is due to disruption of endothelial cell interaction with the extracellular matrix that are necessary for angiogenesis.

Example 6. Endothelial Cell Migration

Invasive cellular processes such as angiogenesis and tumor metastasis also require cellular motility. Thus we evaluated the ability of isolated NC1 domains to support human endothelial cell migration in vitro. These experiments were conducted essentially according to the methods in Brooks et al., J. Clin. Invest. 99:1390-1398 (1997).

The results of these experiments indicate that NC1 $\alpha 2$, $\alpha 3$, and $\alpha 6$ domains can support human endothelial cell migration in vitro, while $\alpha 1$, $\alpha 4$, and $\alpha 5$ domains showed little if any capacity to support endothelial cell migration **(FIG 16)**.

Example 7. Efficacy in Lewis lung in vivo tumor

The above studies indicated that specific domains of collagen type IV can promote cell migration in vitro. Thus, we evaluated the ability of NC1 domains to support endothelial cell migration in vivo.

The α (IV) NC1 domain hexamer, isolated by enzymatic digestion of bovine lens capsule basement membrane by known protocols (Peczon et al., Exp. Eye Res. 30:155-165 (1980)) was tested in the metastatic Lewis lung mouse tumor model using a standard protocol which is considered to be a good model of both metastasis and angiogenesis of lung tumors. (See for example, Teicher et al., Anticancer Res.

18:2567-2573 (1998); Guibaud et al., Anticancer Drugs 8:276-282 (1997); Anderson et al., Cancer Res. 56:715-718 (1996)).

Each study consisted of an untreated control group and six treatment groups. There were ten animals per treatment group with 40 mice in the control. In each study, all treatment was administered intravenously once every 2 days for 7 doses starting one day after tumor inoculation. Dosages of α (IV) NC1 hexamer were either 100 μg/mouse or 200 μg/mouse. In the Lewis lung study, the tumor cell inoculum was 1 $x10^6$ viable cells. All animals were weighed twice a week throughout the study. Starting one day after the last treatment, 5 mice were periodically sacrificed from each control group to measure pulmonary tumor burden. The experiment was terminated at day 14 when the lungs of the control animals had sufficient tumor mass to provide meaningful evaluation. At that time, the lungs of all remaining animals were excised, weighed, and the number of tumor foci greater than 2 mm in diameter counted. The resulting data showed that both dosages of α (IV) NC1 hexamer significantly reduced the number of visible lung metastases (Mann-Whitney Rank Sum Test, $p < 0.05$), with 8 visible lung metastases in the control, vs. 5 (100 μg/mouse) and 4 (200 μg/mouse), and the 100 μg/mouse dosage reduced the lung weights from a median of 520 mg in controls to a median of 462 mg in experimental, while the median lung weight of mice treated with 200 μg/mouse was 620 mg.

Other in vivo studies demonstrated that tumor cell metastasis to the lung can be reduced by 50% or more using intravenous injections of the Type IV collagen domains in murine B16 melanoma, human A375SM melanoma xenografts. Furthermore, injection of the NC1 hexamer also significantly reduced the number of lung tumors in separate Lewis Lung tumor studies.

Example 8. Defining the Integrin Receptor Mediating Cellular Adhesion to the NC1 domains

To define the integrin receptors that mediated cellular adhesion to the NC1 α1 and α2 domains, adhesion assays were performed as described in Example 5 in the presence or absence of function blocking monoclonal antibodies directed to specific integrins (**Figures 18 (α2); Fig. 19 (α1)**). These antibodies were directed against α5ß3 integrin (anti-avb3), the α5ß5 integrin (anti-avb5), the ß1 integrin (b1) (all described in U.S. Patent No. 5,766,591, incorporated by reference herein in its entirety), and monoclonal antibodies directed against the α1 (anti-a1), α2 (anti-a2), and α3 (anti-a3) integrins (purchased from Chemicon, California). These studies indicated that human endothelial cells interact with NC1 α2 domain primarily through αvß5 and αvß3 integrins with variable contribution from ß1 integrins (**Figure 18**). In similar experiments, anti-ß1 integrin antibodies showed a lesser effect on endothelial cell adhesion to NC1 α2, suggesting a lesser contribution of ß1 integrins to this adhesive activity. In contrast, endothelial cell adhesion promoted by NC1 α1 domain was mediated by integrin α3ß1 (**Figure 19**).

Previous studies have demonstrated that RGD-containing antagonists of the αvβ3 receptor can block angiogenesis (U.S. Patent No. 5,766,591), but the instant invention provides the first demonstration of a non-RGD containing antagonist of the αvβ3 integrin that can block angiogenesis. The present study also demonstrates that antagonists of the αvβ5 integrin and the α3β1 integrins can block angiogenesis.

Thus, the instant invention also provides methods and kits for inhibiting angiogenesis, tumor growth and metastasis, and endothelial cell interaction with the extracellular matrix, each method comprising contacting the tumor, animal tissue, or

endothelial cells with antagonists of specific integrin receptors. Specifically, the methods comprise contacting the tumor, animal tissue, or endothelial cells with one or more of the following polypeptide compositions:

(a) a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists; or

(b) a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin; or

(c) a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins; or

(d) a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrins.

We conclude from all of the above studies that angiogenesis, tumor growth and metastasis, and endothelial cell adhesion to the ECM, can be inhibited by isolated, recombinant domains of type IV collagen, or by antagonists of specific integrin receptors. The present invention is thus broadly applicable to a variety of uses which include inhibition of angiogenesis and treatment of diseases and conditions with accompanying undesired angiogenesis, such as solid and blood-borne tumors including but not limited to melanomas, carcinomas, sarcomas, rhabdomyosarcoma, retinoblastoma., Ewing sarcoma, neuroblastoma, osteosarcoma, and leukemia.

The invention is further applicable to treating non-tumorigenic diseases and conditions with accompanying undesired angiogenesis, including but not limited to diabetic retinopathy, rheumatoid arthritis, retinal neovascularization, choroidal neovascularization, macular degeneration., corneal neovascularization, retinopathy of

prematurity., corneal graft rejection, neovascular glaucoma., retrolental fibroplasia, epidemic keratoconjunctivitis, Vitamin A deficiency, contact lens overwear, atopic keratitis, superior limbic keratitis, pterygium keratitis sicca, sogrens, acne rosacea, phylectenulosis, syphilis, Mycobacteria infections, lipid degeneration, chemical burns, bacterial ulcers, fungal ulcers, Herpes simplex infections, Herpes zoster infections, protozoan infections, Kaposi's sarcoma, Mooren ulcer, Terrien's marginal degeneration, marginal keratolysis, traum, systemic lupus, polyarteritis, Wegeners sarcoidosis, scleritis, Steven's Johnson disease, radial keratotomy, sickle cell anemia, sarcoid, pseudoxanthoma elasticum, Pagets disease, vein occlusion, artery occulsion, carotid obstructive disease, chronic uveitis, chronic vitritis, Lyme's disease, Eales disease, Bechets disease, myopia, optic pits, Stargarts disease, pars planitis, chronic retinal detachment, hyperviscosity syndromes, toxoplasmosis, post-laser complications, abnormal proliferation of fibrovascular tissue, hemangiomas, Osler-Weber-Rendu, acquired immune deficiency syndrome, ocular neovascular disease, osteoarthritis, chronic inflammation, Crohn's disease, ulceritive colitis, psoriasis., atherosclerosis, and pemphigoid. See U.S. Patent No. 5,712,291)

The invention is also broadly applicable to methods for inhibiting tumor growth and metastasis, reduction of scar tissue formation, reduction of complications due to cell adhesion in organ transplants, and the inhibition of lymphocyte adhesion and mobility.

While the fundamental novel features of the invention have been shown and described, it will be understood that various omissions, substitutions, and changes in the form and details illustrated may be made by those skilled in the art without departing from the spirit of the invention. For example, various modifications,

additions. and/or substitutions can be made to the type IV collagen α monomer chains that would be encompassed by the invention.

We claim

1. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

2. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

3. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

4. A method for inhibiting angiogenesis in an animal tissue comprising contacting the tumor or animal tissue with an amount effective to inhibit angiogenesis of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

5. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta 3$ antagonists.

6. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha v\beta 5$ integrin.

7. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial

cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\alpha3\beta1$ integrin.

8. A method for inhibiting endothelial cell adhesion to extracellular matrix, comprising contacting the endothelial cell with an amount effective to inhibit endothelial cell adhesion to extracellular matrix of a polypeptide composition comprising one or more antagonists of $\beta1$ integrins.

9. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta3$ antagonists.

10. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha v\beta5$ integrin.

11. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\alpha3\beta1$ integrin.

12. A method for inhibiting tumor metastasis in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor metastasis of a polypeptide composition comprising one or more antagonists of $\beta1$ integrins.

13. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more non-RGD containing integrin $\alpha v\beta3$ antagonists.

14. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha v\beta5$ integrin.

15. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\alpha 3\beta 1$ integrin.

16. A method for inhibiting tumor growth in tissue comprising contacting the tumor or tissue with an amount effective to inhibit tumor growth of a polypeptide composition comprising one or more antagonists of $\beta 1$ integrins.

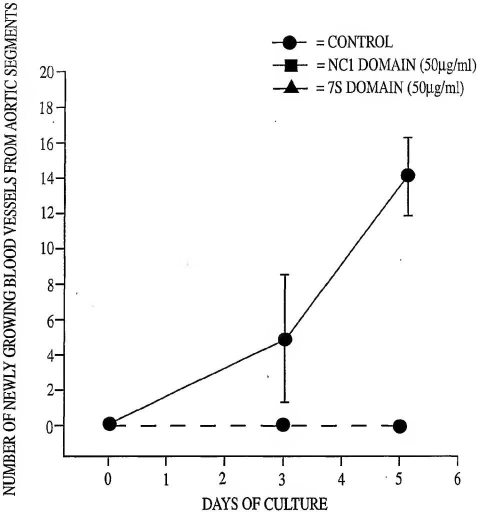

NUMBER OF NEWLY GROWING BLOOD VESSELS FROM AORTIC SEGMENTS
= CONTROL
= NC1 DOMAIN (50µg/ml)
= 7S DOMAIN (50µg/ml)
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
DAYS OF CULTURE

FIG. 1

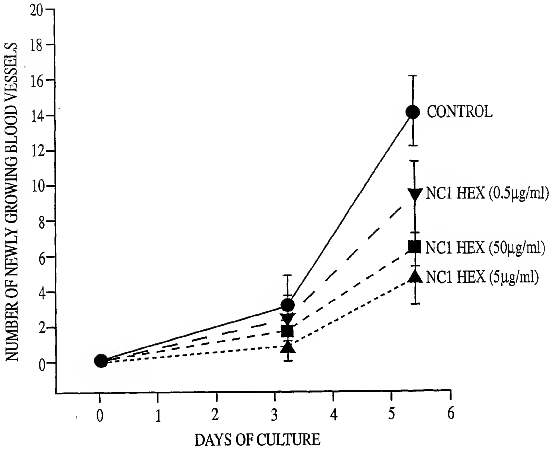

NUMBER OF NEWLY GROWING BLOOD VESSELS
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
DAYS OF CULTURE
CONTROL
NC1 HEX (0.5μg/ml)
NC1 HEX (50μg/ml)
NC1 HEX (5μg/ml)

FIG. 2

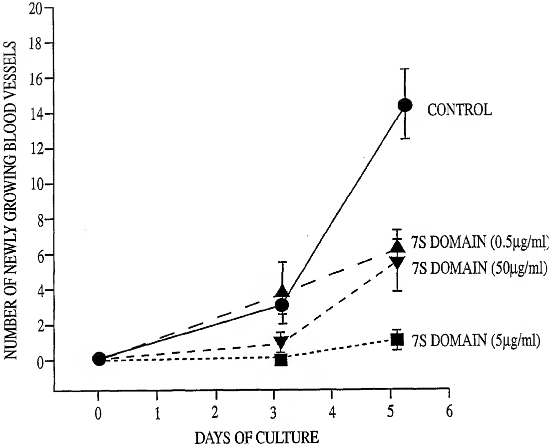
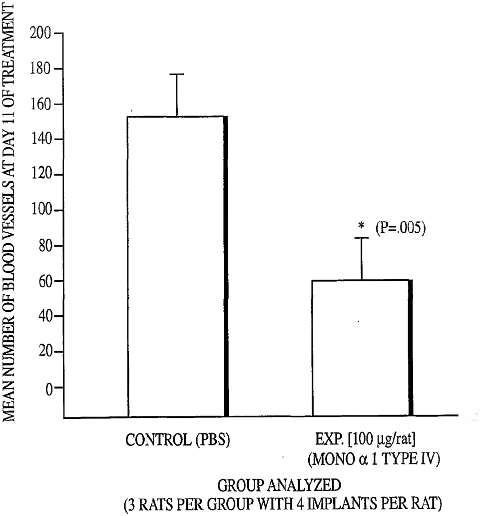

NUMBER OF NEWLY GROWING BLOOD VESSELS
20
18
16
14
12
10
8
6
4
2
0
0
1
2
3
4
5
6
DAYS OF CULTURE
CONTROL
7S DOMAIN (0.5µg/ml)
7S DOMAIN (50µg/ml)
7S DOMAIN (5µg/ml)

FIG. 3

CONTROL
FIG. 4a

7S DOMAIN (50μg/ml)
FIG. 4b

NC1 DOMAIN (50μg/ml)
FIG. 4c



MEAN NUMBER OF BLOOD VESSELS AT DAY 11 OF TREATMENT
200
180
160
140
120
100
80
60
40
20
0
* (P=.005)
CONTROL (PBS)
EXP. [100 μg/rat]
(MONO α 1 TYPE IV)
GROUP ANALYZED
(3 RATS PER GROUP WITH 4 IMPLANTS PER RAT)

FIG. 5

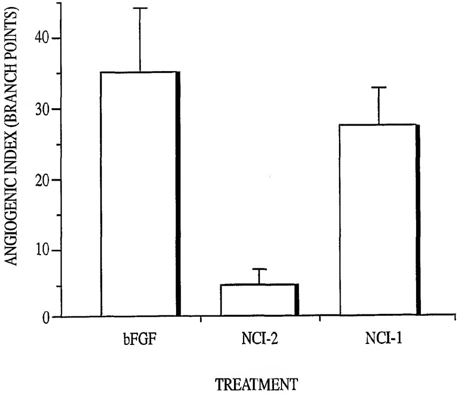

ANGIOGENIC INDEX (BRANCH POINTS)
40
30
20
10
0
bFGF
NCI-2
NCI-1
TREATMENT

FIG. 6

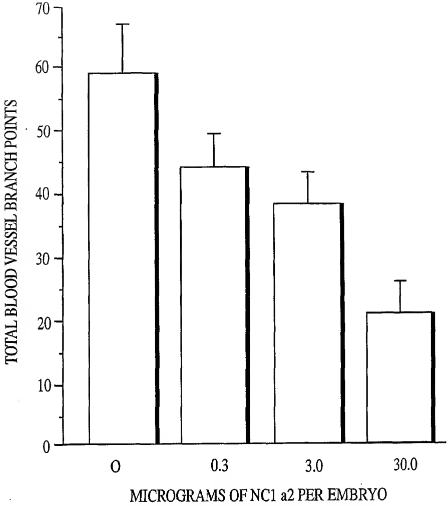

70
60
50
40
30
20
10
0
TOTAL BLOOD VESSEL BRANCH POINTS
O
0.3
3.0
30.0
MICROGRAMS OF NC1 a2 PER EMBRYO

FIG. 7

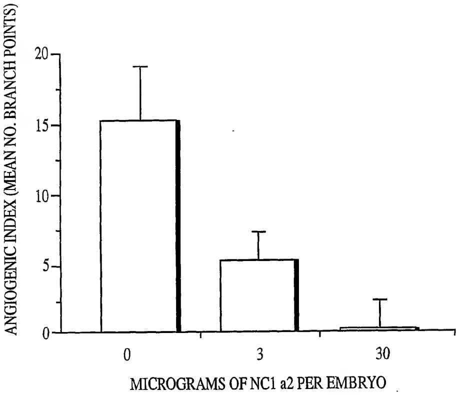

ANGIOGENIC INDEX (MEAN NO. BRANCH POINTS)
20
15
10
5
0
0
3
30
MICROGRAMS OF NC1 a2 PER EMBRYO

FIG. 8

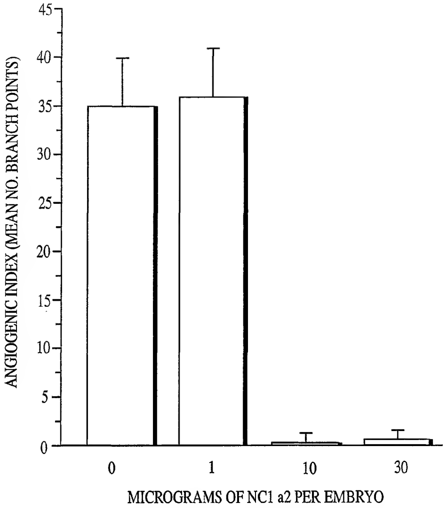
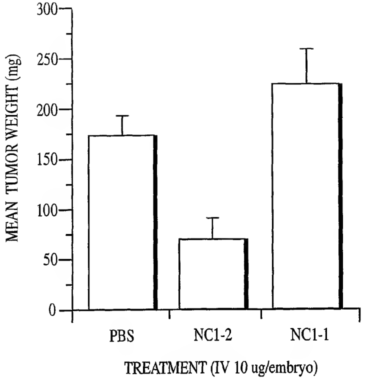

ANGIOGENIC INDEX (MEAN NO. BRANCH POINTS)
45
40
35
30
25
20
15
10
5
0
0
1
10
30
MICROGRAMS OF NC1 a2 PER EMBRYO

FIG. 9



MEAN TUMOR WEIGHT (mg)
300
250
200
150
100
50
0
PBS
NC1-2
NC1-1
TREATMENT (IV 10 ug/embryo)

FIG. 10

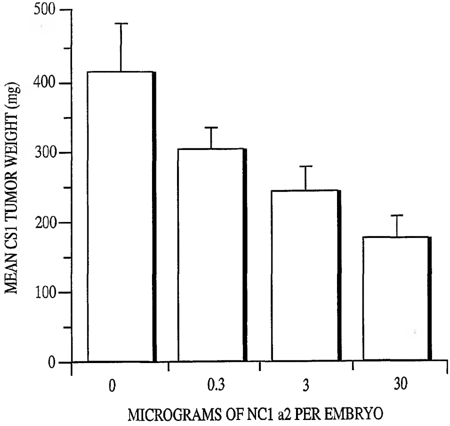

500
400
300
200
100
0
MEAN CS1 TUMOR WEIGHT (mg)
0
0.3
3
30
MICROGRAMS OF NC1 a2 PER EMBRYO

FIG. 11

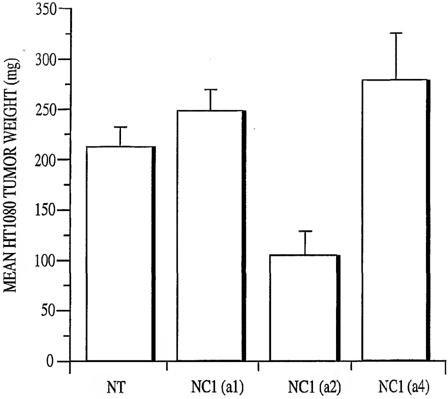

MEAN HT1080 TUMOR WEIGHT (mg)
350
300
250
200
150
100
50
0
NT
NC1 (a1)
NC1 (a2)
NC1 (a4)

FIG. 12

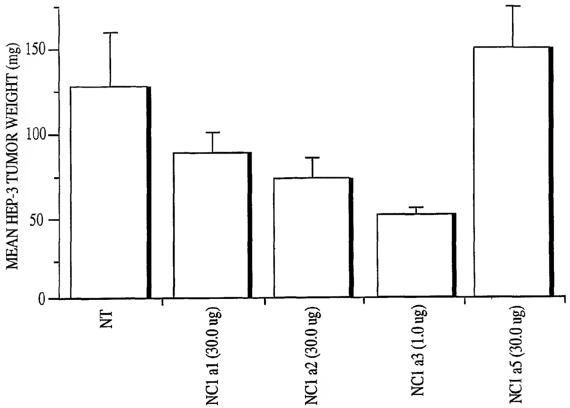

MEAN HEP-3 TUMOR WEIGHT (mg)
150
100
50
0
NT
NC1 a1 (30.0 ug)
NC1 a2 (30.0 ug)
NC1 a3 (1.0 ug)
NC1 a5 (30.0 ug)

FIG. 13

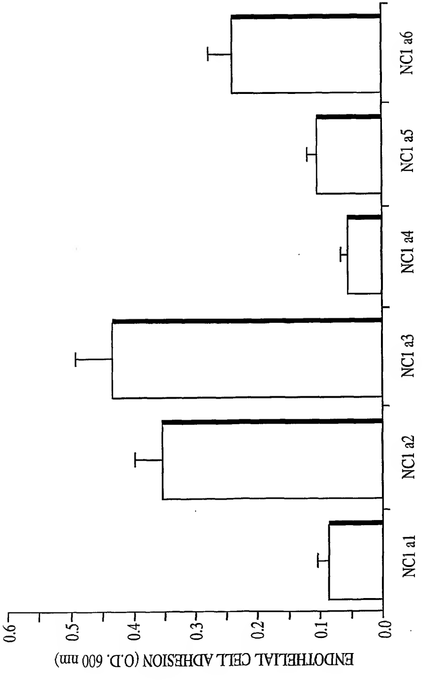

ENDOTHELIAL CELL ADHESION (O.D. 600 nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
NC1 a1
NC1 a2
NC1 a3
NC1 a4
NC1 a5
NC1 a6

FIG. 14

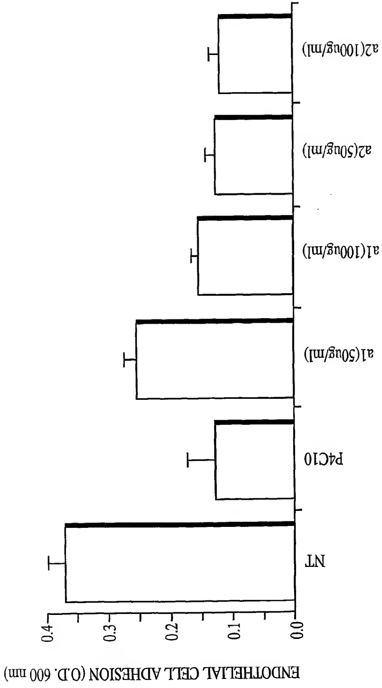

ENDOTHELIAL CELL ADHESION (O.D. 600 nm)
0.0
0.1
0.2
0.3
0.4
NT
P4C10
a1(50ug/ml)
a1(100ug/ml)
a2(50ug/ml)
a2(100ug/ml)

FIG. 15

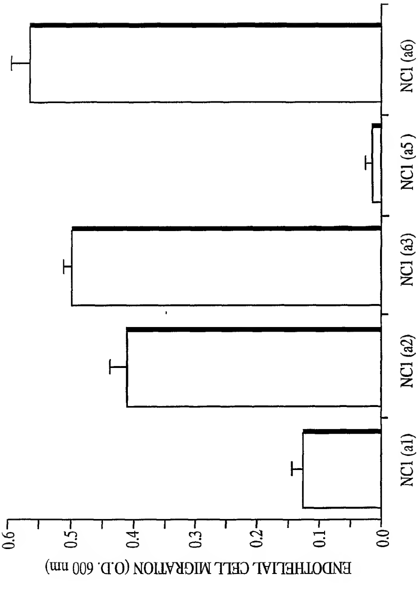

ENDOTHELIAL CELL MIGRATION (O.D. 600 nm)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
NC1 (a1)
NC1 (a2)
NC1 (a3)
NC1 (a5)
NC1 (a6)

FIG. 16

A. α1(IV)NC1

```
900       910       920       930       940       950
|         |         |         |         |         |
CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                         M  R  A  W  I  F  F

960       970       980       990       1000      1010
|         |         |         |         |         |
CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT
 L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020      1030      1040      1050      1060      1070
|         |         |         |         |         |
GACAAGCTAGCATCTGTTGATCACGGCTTCCTTGTGACCAGGCATAGTCAAACAATAGAT
 D  K  L  A  S  V  D  H  G  F  L  V  T  R  H  S  Q  T  I  D

1080      1090      1100      1110      1120      1130
|         |         |         |         |         |
GACCCACAGTGTCCTTCTGGGACCAAAATTCTTTACCACGGGTACTCTTTGCTCTACGTG
 D  P  Q  C  P  S  G  T  K  I  L  Y  H  G  Y  S  L  L  Y  V

1140      1150      1160      1170      1180      1190
|         |         |         |         |         |
CAAGGCAATGAACGGGCCCATGGCCAGGACTTGGGCACGGCCGGCAGCTGCCTGCGCAAG
 Q  G  N  E  R  A  H  G  Q  D  L  G  T  A  G  S  C  L  R  K

1200      1210      1220      1230      1240      1250
|         |         |         |         |         |
TTCAGCACAATGCCCTTCCTGTTCTGCAATATTAACAACGTGTGCAACTTTGCATCACGA
 F  S  T  M  P  F  L  F  C  N  I  N  N  V  C  N  F  A  S  R

1260      1270      1280      1290      1300      1310
|         |         |         |         |         |
AATGACTACTCGTACTGGCTGTCCACCCCTGAGCCCATGCCCATGTCAATGGCACCCATC
 N  D  Y  S  Y  W  L  S  T  P  E  P  M  P  M  S  M  A  P  I

1320      1330      1340      1350      1360      1370
|         |         |         |         |         |
ACGGGGGAAAACATAAGACCATTTATTAGTAGGTGTGCTGTGTGTGAGGCGCCTGCCATG
 T  G  E  N  I  R  P  F  I  S  R  C  A  V  C  E  A  P  A  M

1380      1390      1400      1410      1420      1430
|         |         |         |         |         |
GTGATGGCCGTGCACAGCCAGACCATTCAGATCCCACCGTGCCCCAGCGGGTGGTCCTCG
 V  M  A  V  H  S  Q  T  I  Q  I  P  P  C  P  S  G  W  S  S

1440      1450      1460      1470      1480      1490
|         |         |         |         |         |
CTGTGGATCGGCTACTCTTTTGTGATGCACACCAGCGCTGGTGCAGAAGGCTCTGGCCAA
 L  W  I  G  Y  S  F  V  M  H  T  S  A  G  A  E  G  S  G  Q

1500      1510      1520      1530      1540      1550
|         |         |         |         |         |
GCCCTGGCGTCCCCCGGCTCCTGCCTGGAGGAGTTTAGAAGTGCGCCATTCATCGAGTGT
```

FIG. 17a

```
    A  L  A  S  P  G  S  C  L  E  E  F  R  S  A  P  F  I  E  C

 1560      1570      1580      1590      1600      1610
    |         |         |         |         |         |
   CACGGCCGTGGGACCTGCAATTACTACGCAAACGCTTACAGCTTTTGGCTCGCCACCATA
    H  G  R  G  T  C  N  Y  Y  A  N  A  Y  S  F  W  L  A  T  I

 1620      1630      1640      1650      1660      1670
    |         |         |         |         |         |
   GAGAGGAGCGAGATGTTCAAGAAGCCTACGCCGTCCACCTTGAAGGCAGGGGAGCTGCGC
    E  R  S  E  M  F  K  K  P  T  P  S  T  L  K  A  G  E  L  R

 1680      1690      1700      1710      1720      1730
    |         |         |         |         |         |
   ACGCACGTCAGCCGCTGCCAAGTCTGTATGAGAAGAACATAATGAAGCCTGACTCAGCTA
    T  H  V  S  R  C  Q  V  C  M  R  R  T  -  -

1740      1750      1760      1770      1780      1790
    |         |         |         |         |         |
   CCGCGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTG
```

FIG. 17a

B. α2(IV)NC1

```
900       910       920       930       940       950
|         |         |         |         |         |
CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                         M  R  A  W  I  F  F

960       970       980       990       1000      1010
|         |         |         |         |         |
CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT
 L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020      1030      1040      1050      1060      1070
|         |         |         |         |         |
GACAAGCTAGCCGTCAGCATCGGCTACCTCCTGGTGAAGCACAGCCAGACGGACCAGGAG
 D  K  L  A  V  S  I  G  Y  L  L  V  K  H  S  Q  T  D  Q  E

1080      1090      1100      1110      1120      1130
|         |         |         |         |         |
CCCATGTGCCCGGTGGGCATGAACAAACTCTGGAGTGGATACAGCCTGCTGTACTTCGAG
 P  M  C  P  V  G  M  N  K  L  W  S  G  Y  S  L  L  Y  F  E

1140      1150      1160      1170      1180      1190
|         |         |         |         |         |
GGCCAGGAGAAGGCGCACAACCAGGACCTGGGGCTGGCGGGCTCCTGCCTGGCGCGGTTC
 G  Q  E  K  A  H  N  Q  D  L  G  L  A  G  S  C  L  A  R  F

1200      1210      1220      1230      1240      1250
|         |         |         |         |         |
AGCACCATGCCCTTCCTGTACTGCAACCCTGGTGATGTCTGCTACTATGCCAGCCGGAAC
 S  T  M  P  F  L  Y  C  N  P  G  D  V  C  Y  Y  A  S  R  N

1260      1270      1280      1290      1300      1310
|         |         |         |         |         |
GACAAGTCCTACTGGCTCTCTACCACTGCGCCGCTGCCCATGATGCCCGTGGCCGAGGAC
 D  K  S  Y  W  L  S  T  T  A  P  L  P  M  M  P  V  A  E  D

1320      1330      1340      1350      1360      1370
|         |         |         |         |         |
GAGATCAAGCCCTACATCAGCCGCTGTTCTGTGTGTGAGGCCCCGGCCATCGCCATCGCG
 E  I  K  P  Y  I  S  R  C  S  V  C  E  A  P  A  I  A  I  A

1380      1390      1400      1410      1420      1430
|         |         |         |         |         |
GTCCACAGTCAGGATGTCTCCATCCCACACTGCCCAGCTGGGTGGCGGAGTTTGTGGATC
 V  H  S  Q  D  V  S  I  P  H  C  P  A  G  W  R  S  L  W  I

1440      1450      1460      1470      1480      1490
|         |         |         |         |         |
GGATATTCCTTCCTCATGCACACGGCGGCGGGAGACGAAGGCGGTGGCCAATCACTGGTG
 G  Y  S  F  L  M  H  T  A  A  G  D  E  G  G  G  Q  S  L  V

1500      1510      1520      1530      1540      1550
```

FIG. 17b

```
          |         |         |         |         |         |
   TCACCGGGCAGCTGTCTAGAGGACTTCCGCGCCACACCATTCATCGAATGCAATGGAGGC
    S  P  G  S  C  L  E  D  F  R  A  T  P  F  I  E  C  N  G  G

1560      1570      1580      1590      1600      1610
   |         |         |         |         |         |
   CGCGGCACCTGCCACTACTACGCCAACAAGTACAGCTTCTGGCTGACCACCATTCCCGAG
    R  G  T  C  H  Y  Y  A  N  K  Y  S  F  W  L  T  T  I  P  E

1620      1630      1640      1650      1660      1670
   |         |         |         |         |         |
   CAGAGCTTCCAGGGCTCGCCCTCCGCCGACACGCTCAAGGCCGGCCTCATCCGCACACAC
    Q  S  F  Q  G  S  P  S  A  D  T  L  K  A  G  L  I  R  T  H

1680      1690      1700      1710      1720      1730
   |         |         |         |         |         |
   ATCAGCCGCTGCCAGGTGTGCATGAAGAACCTGTGAGCCGGCGCGTGCCAGGGCCCTATT
    I  S  R  C  Q  V  C  M  K  N  L  -

1740      1750      1760      1770      1780      1790
   |         |         |         |         |         |
   CTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGC
```

FIG. 17b

C. α3(IV)NC1

```
900        910        920        930        940        950
|          |          |          |          |          |
CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                         M  R  A  W  I  F  F

960        970        980        990        1000       1010
|          |          |          |          |          |
CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCGCTAGCCGACTACAAGGACGACGAT
 L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020       1030       1040       1050       1060       1070
|          |          |          |          |          |
GACAAACGTGGAGACAGTGGATCACCTGCAACCTGGACAACGAGAGGCTTTGTCTTCACC
 D  K  R  G  D  S  G  S  P  A  T  W  T  T  R  G  F  V  F  T

1080       1090       1100       1110       1120       1130
|          |          |          |          |          |
CGACACAGTCAAACCACAGCAATTCCTTCATGTCCAGAGGGGACAGTGCCACTCTACAGT
 R  H  S  Q  T  T  A  I  P  S  C  P  E  G  T  V  P  L  Y  S

1140       1150       1160       1170       1180       1190
|          |          |          |          |          |
GGGTTTTCTTTTCTTTTTGTACAAGGAAATCAACGAGCCCACGGACAAGACCTTGGAACT
 G  F  S  F  L  F  V  Q  G  N  Q  R  A  H  G  Q  D  L  G  T

1200       1210       1220       1230       1240       1250
|          |          |          |          |          |
CTTGGCAGCTGCCTGCAGCGATTTACCACAATGCCATTCTTATTCTGCAATGTCAATGAT
 L  G  S  C  L  Q  R  F  T  T  M  P  F  L  F  C  N  V  N  D

1260       1270       1280       1290       1300       1310
|          |          |          |          |          |
GTATGTAATTTTGCATCTCGAAATGATTATTCATACTGGCTGTCAACACCAGCTCTGATG
 V  C  N  F  A  S  R  N  D  Y  S  Y  W  L  S  T  P  A  L  M

1320       1330       1340       1350       1360       1370
|          |          |          |          |          |
CCAATGAACATGGCTCCCATTACTGGCAGAGCCCTTGAGCCTTATATAAGCAGATGCACT
 P  M  N  M  A  P  I  T  G  R  A  L  E  P  Y  I  S  R  C  T

1380       1390       1400       1410       1420       1430
|          |          |          |          |          |
GTTTGTGAAGGTCCTGCGATCGCCATAGCCGTTCACAGCCAAACCACTGACATTCCTCCA
 V  C  E  G  P  A  I  A  I  A  V  H  S  Q  T  T  D  I  P  P

1440       1450       1460       1470       1480       1490
|          |          |          |          |          |
TGTCCTCACGGCTGGATTTCTCTCTGGAAAGGATTTTCATTCATCATGTTCACAAGTGCA
 C  P  H  G  W  I  S  L  W  K  G  F  S  F  I  M  F  T  S  A

1500       1510       1520       1530       1540       1550
```

FIG. 17c

```
|        |        |        |        |        |
GGTTCTGAGGGCGCCGGGCAAGCACTGGCCTCCCCCGGCTCCTGCCTGGAAGAATTCCGA
 G  S  E  G  A  G  Q  A  L  A  S  P  G  S  C  L  E  E  F  R

1560      1570      1580      1590      1600      1610
|         |         |         |         |         |
GCCAGCCCATTTCTAGAATGTCATGGAAGAGGAACGTGCAACTACTATTCAAATTCCTAC
 A  S  P  F  L  E  C  H  G  R  G  T  C  N  Y  Y  S  N  S  Y

1620      1630      1640      1650      1660      1670
|         |         |         |         |         |
AGTTTCTGGCTGGCTTCATTAAACCCAGAAAGAATGTTCAGAAAGCCTATTCCATCAACT
 S  F  W  L  A  S  L  N  P  E  R  M  F  R  K  P  I  P  S  T

1680      1690      1700      1710      1720      1730
|         |         |         |         |         |
GTGAAAGCTGGGGAATTAGAAAAAATAATAAGTCGCTGTCAGGTGTGCATGAAGAAAAGA
 V  K  A  G  E  L  E  K  I  I  S  R  C  Q  V  C  M  K  K  R

1740      1750      1760      1770      1780      1790
|         |         |         |         |         |
CACTGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGAC

 H  -
```

FIG. 17c

D. α4(IV)NC1

```
 900       910       920       930       940       950
  |         |         |         |         |         |
  CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                           M  R  A  W  I  F  F

 960       970       980       990      1000      1010
  |         |         |         |         |         |
  CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCGCTAGCCGACTACAAGGACGACGAT
   L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020      1030      1040      1050      1060      1070
  |         |         |         |         |         |
  GACAAGCCTGGATACCTCGGTGGCTTCCTCCTGGTTCTCCACAGTCAGACGGACCAGGAG
   D  K  P  G  Y  L  G  G  F  L  L  V  L  H  S  Q  T  D  Q  E

1080      1090      1100      1110      1120      1130
  |         |         |         |         |         |
  CCCACCTGCCCCCTGGGCATGCCCAGGCTCTGGACTGGGTATAGTCTGTTATACCTGGAA
   P  T  C  P  L  G  M  P  R  L  W  T  G  Y  S  L  L  Y  L  E

1140      1150      1160      1170      1180      1190
  |         |         |         |         |         |
  GGGCAAGAGAAAGCTCACAATCAAGACCTTGGTCTGGCAGGGTCTTGCCTTCCCGTATTT
   G  Q  E  K  A  H  N  Q  D  L  G  L  A  G  S  C  L  P  V  F

1200      1210      1220      1230      1240      1250
  |         |         |         |         |         |
  AGCACGCTGCCCTTTGCCTACTGCAACATCCACCAGGTGTGCCACTATGCCCAGAGAAAC
   S  T  L  P  F  A  Y  C  N  I  H  Q  V  C  H  Y  A  Q  R  N

1260      1270      1280      1290      1300      1310
  |         |         |         |         |         |
  GACAGATCCTACTGGCTGGCCAGCGCTGCGCCCCTCCCCATGATGCCACTCTCTGAAGAG
   D  R  S  Y  W  L  A  S  A  A  P  L  P  M  M  P  L  S  E  E

1320      1330      1340      1350      1360      1370
  |         |         |         |         |         |
  GCGATCCGCCCCTATGTCAGCCGCTGTGCGGTATGCGAGGCCCCGGCCCAGGCGGTGGCG
   A  I  R  P  Y  V  S  R  C  A  V  C  E  A  P  A  Q  A  V  A

1380      1390      1400      1410      1420      1430
  |         |         |         |         |         |
  GTGCACAGCCAGGACCAGTCCATCCCCCCATGTCCGCAGACCTGGAGGAGCCTCTGGATC
   V  H  S  Q  D  Q  S  I  P  P  C  P  Q  T  W  R  S  L  W  I

1440      1450      1460      1470      1480      1490
  |         |         |         |         |         |
  GGGTATTCATTCCTGATGCACACAGGAGCTGGGGACCAAGGAGGAGGGCAGGCCCTTATG
   G  Y  S  F  L  M  H  T  G  A  G  D  Q  G  G  G  Q  A  L  M

1500      1510      1520      1530      1540      1550
```

FIG. 17d

```
TCACCTGGCAGCTGCCTGGAAGATTTCAGAGCAGCACCATTCCTTGAATGCCAGGGCCGG
 S  P  G  S  C  L  E  D  F  R  A  A  P  F  L  E  C  Q  G  R

1560      1570      1580      1590      1600      1610
CAGGGAACTTGCCACTTTTTCGCAAATAAGTATAGCTTCTGGCTCACAACGGTGAAAGCA
 Q  G  T  C  H  F  F  A  N  K  Y  S  F  W  L  T  T  V  K  A

1620      1630      1640      1650      1660      1670
GACTTGCAGTTTTCCTCTGCTCCAGCACCAGACACCTTAAAAGAAAGCCAGGCCCAACGC
 D  L  Q  F  S  S  A  P  A  P  D  T  L  K  E  S  Q  A  Q  R

1680      1690      1700      1710      1720      1730
CAGAAAATCAGCCGGTGCCAGGTCTGCGTGAAGTATAGCTAGGGGCCCTATTCTATAGTG
 Q  K  I  S  R  C  Q  V  C  V  K  Y  S  -

1740      1750      1760      1770      1780      1790
TCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTCTAGTTGCCAGCCATC
```

FIG. 17d

E. α5(IV)NC1

```
 900        910       920       930       940       950
  |          |         |         |         |         |
  CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                              M  R  A  W  I  F  F

 960        970       980       990      1000      1010
  |          |         |         |         |         |
  CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCGCTAGCTGACTACAAGGACGACGAT
   L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020       1030      1040      1050      1060      1070
  |          |         |         |         |         |
  GACAAAGGTCCCCCTGGAACCTCCTCTGTTGCACATGGATTTCTTATTACACGCCACAGC
   D  K  G  P  P  G  T  S  S  V  A  H  G  F  L  I  T  R  H  S

1080       1090      1100      1110      1120      1130
  |          |         |         |         |         |
  CAGACAACGGATGCACCACAATGCCCACAGGGAACACTTCAGGTCTATGAAGGCTTTTCT
   Q  T  T  D  A  P  Q  C  P  Q  G  T  L  Q  V  Y  E  G  F  S

1140       1150      1160      1170      1180      1190
  |          |         |         |         |         |
  CTCCTGTATGTACAAGGAAATAAAAGAGCCCACGGTCAAGACTTGGGGACGGCTGGCAGC
   L  L  Y  V  Q  G  N  K  R  A  H  G  Q  D  L  G  T  A  G  S

1200       1210      1220      1230      1240      1250
  |          |         |         |         |         |
  TGCCTTCGTCGCTTTAGTACCATGCCTTTCATGTTCTGCAACATCAATAATGTTTGCAAC
   C  L  R  R  F  S  T  M  P  F  M  F  C  N  I  N  N  V  C  N

1260       1270      1280      1290      1300      1310
  |          |         |         |         |         |
  TTTGCTTCAAGAAATGACTATTCTTACTGGCTCTCTACCCCAGAGCCCATGCCAATGAGC
   F  A  S  R  N  D  Y  S  Y  W  L  S  T  P  E  P  M  P  M  S

1320       1330      1340      1350      1360      1370
  |          |         |         |         |         |
  ATGCAACCCCTAAAGGGCCAGAGCATCCAGCCATTCATTAGTCGATGTGCAGTATGTGAA
   M  Q  P  L  K  G  Q  S  I  Q  P  F  I  S  R  C  A  V  C  E

1380       1390      1400      1410      1420      1430
  |          |         |         |         |         |
  GCTCCAGCTGTGGTGATCGCAGTTCACAGTCAGACGATCCAGATTCCCCATTGTCCTCAG
   A  P  A  V  V  I  A  V  H  S  Q  T  I  Q  I  P  H  C  P  Q

1440       1450      1460      1470      1480      1490
  |          |         |         |         |         |
  GGATGGGATTCTCTGTGGATTGGTTATTCCTTCATGATGCATACAAGTGCAGGGGCAGAA
   G  W  D  S  L  W  I  G  Y  S  F  M  M  H  T  S  A  G  A  E

1500       1510      1520      1530      1540      1550
```

FIG. 17e

```
|         |         |         |         |         |
GGCTCAGGTCAAGCCCTAGCCTCCCCTGGTTCCTGCTTGGAAGAGTTTCGTTCAGCTCCC
 G  S  G  Q  A  L  A  S  P  G  S  C  L  E  E  F  R  S  A  P

1560      1570      1580      1590      1600      1610
|         |         |         |         |         |
TTCATCGAATGTCATGGAGGGGTACCTGTAACTACTATGCCAACTCCTACAGCTTTTGG
 F  I  E  C  H  G  R  G  T  C  N  Y  Y  A  N  S  Y  S  F  W

1620      1630      1640      1650      1660      1670
|         |         |         |         |         |
CTGGCAACTGTAGATGTGTCAGACATGTTCAGTAAACCTCAGTCAGAAACGCTGAAAGCA
 L  A  T  V  D  V  S  D  M  F  S  K  P  Q  S  E  T  L  K  A

1680      1690      1700      1710      1720      1730
|         |         |         |         |         |
GGAGACTTGAGGACACGAATTAGCCGATGTCAAGTGTGCATGAAGAGGACATAACGCGGC
 G  D  L  R  T  R  I  S  R  C  Q  V  C  M  K  R  T  -

1740      1750      1760      1770      1780      1790
|         |         |         |         |         |
CGCTCGAGCATGCATCTAGAGGGCCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGC
```

FIG. 17e

F. α6 (IV) NC1

```
 900        910        920        930        940        950
  |          |          |          |          |          |
  CTGCCGCCTGCCTGCCTGCCACTGAGGGTTCCCAGCACCATGAGGGCCTGGATCTTCTTT
                                          M  R  A  W  I  F  F

 960        970        980        990       1000       1010
  |          |          |          |          |          |
  CTCCTTTGCCTGGCCGGGAGGGCTCTGGCAGCCCCACTAGCCGACTACAAGGACGACGAT
   L  L  C  L  A  G  R  A  L  A  A  P  L  A  D  Y  K  D  D  D

1020       1030       1040       1050       1060       1070
  |          |          |          |          |          |
  GACAAGCTAGCGAGCATGAGAGTGGGCTACACGTTGGTAAAGCACAGCCAGTCGGAACAG
   D  K  L  A  S  M  R  V  G  Y  T  L  V  K  H  S  Q  S  E  Q

1080       1090       1100       1110       1120       1130
  |          |          |          |          |          |
  GTGCCCCCGTGTCCCATCGGGATGAGCCAGCTGTGGGTGGGGTACAGCTTACTGTTTGTG
   V  P  P  C  P  I  G  M  S  Q  L  W  V  G  Y  S  L  L  F  V

1140       1150       1160       1170       1180       1190
  |          |          |          |          |          |
  GAGGGGCAAGAGAAAGCCCACAACCAGGACCTGGGCTTTGCTGGCTCCTGTCTGCCCCGC
   E  G  Q  E  K  A  H  N  Q  D  L  G  F  A  G  S  C  L  P  R

1200       1210       1220       1230       1240       1250
  |          |          |          |          |          |
  TTCAGCACCATGCCCTTCATCTACTGCAACATCAACGAGGTGTGCCACTATGCCAGGCGC
   F  S  T  M  P  F  I  Y  C  N  I  N  E  V  C  H  Y  A  R  R

1260       1270       1280       1290       1300       1310
  |          |          |          |          |          |
  AATGATAAATCTTACTGGCTCTCCACTACCGCCCCTATCCCCATGATGCCCGTCAGCCAG
   N  D  K  S  Y  W  L  S  T  T  A  P  I  P  M  M  P  V  S  Q

1320       1330       1340       1350       1360       1370
  |          |          |          |          |          |
  ACCCAGATTCCCCAGTACATCAGCCGCTGCTCTGTGTGTGAGGCACCCTCGCAAGCCATT
   T  Q  I  P  Q  Y  I  S  R  C  S  V  C  E  A  P  S  Q  A  I

1380       1390       1400       1410       1420       1430
  |          |          |          |          |          |
  GCTGTGCACAGCCAGGACATCACCATCCCGCAGTGCCCCCTGGGCTGGCGCAGCCTCTGG
   A  V  H  S  Q  D  I  T  I  P  Q  C  P  L  G  W  R  S  L  W

1440       1450       1460       1470       1480       1490
  |          |          |          |          |          |
  ATTGGGTACTCTTTCCTCATGCACACTGCCGCTGGTGCCGAGGGTGGAGGCCAGTCCCTG
   I  G  Y  S  F  L  M  H  T  A  A  G  A  E  G  G  G  Q  S  L

1500       1510       1520       1530       1540       1550
```

FIG. 17f

```
GTCTCACCTGGCTCCTGCCTAGAGGACTTTCGGGCCACTCCTTTCATCGAATGCAGTGGT
 V  S  P  G  S  C  L  E  D  F  R  A  T  P  F  I  E  C  S  G

1560      1570      1580      1590      1600      1610
GCCCGAGGCACCTGCCACTACTTTGCAAACAAGTACAGTTTCTGGTTGACCACAGTGGAG
 A  R  G  T  C  H  Y  F  A  N  K  Y  S  F  W  L  T  T  V  E

1620      1630      1640      1650      1660      1670
GAGAGGCAGCAGTTTGGGGAGTTGCCTGTGTCTGAAACGCTGAAAGCTGGGCAGCTCCAC
 E  R  Q  Q  F  G  E  L  P  V  S  E  T  L  K  A  G  Q  L  H

1680      1690      1700      1710      1720      1730
ACTCGAGTCAGTCGCTGCCAGGTGTGTATGAAAAGCCTGTAGGGTGGCACCTGCCACGGG
 T  R  V  S  R  C  Q  V  C  M  K  S  L  -

1740      1750      1760      1770      1780      1790
CCCTATTCTATAGTGTCACCTAAATGCTAGAGCTCGCTGATCAGCCTCGACTGTGCCTTC
```

FIG. 17f

FIG. 18

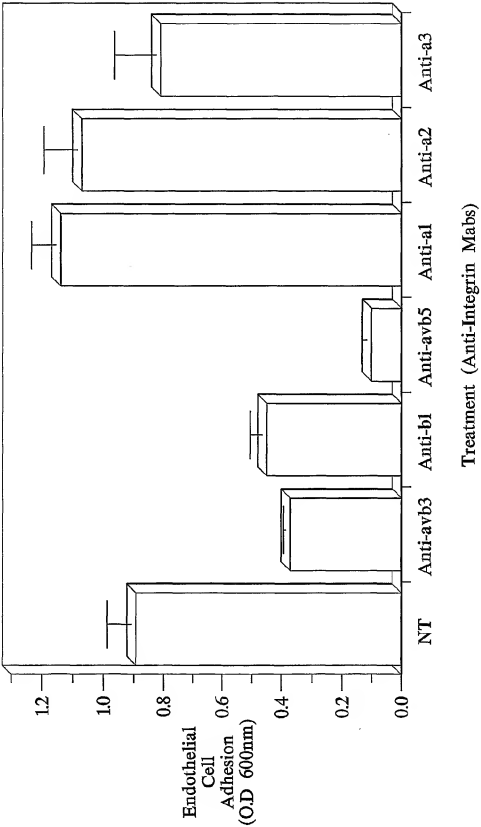
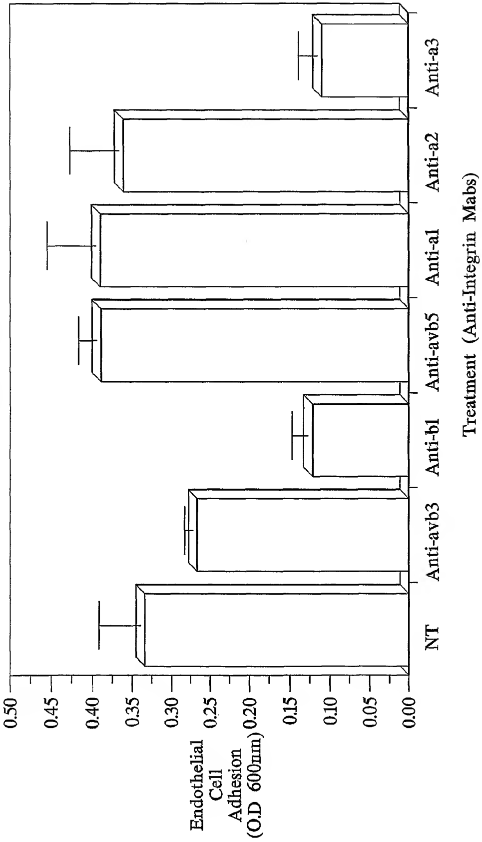

Endothelial Cell Adhesion (O.D 600nm)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
NT
Anti-avb3
Anti-b1
Anti-avb5
Anti-a1
Anti-a2
Anti-a3
Treatment (Anti-Integrin Mabs)

FIG. 19


Endothelial Cell Adhesion (O.D 600nm)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
NT
Anti-avb3
Anti-b1
Anti-avb5
Anti-a1
Anti-a2
Anti-a3
Treatment (Anti-Integrin Mabs)

SEQUENCE LISTING

<110> Biostratum, Inc. et al.

<120> Methods to Inhibit Angiogenesis and Tumor Growth

<130> 94525K2

<140> To be assigned
<141> Herewith

<160> 12

<170> PatentIn Ver. 2.0

<210> 1
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(819)

<400> 1
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc 54
Met Arg Ala Trp Ile
1 5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc 102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
10 15 20

gac tac aag gac gac gat gac aag cta gca tct gtt gat cac ggc ttc 150
Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser Val Asp His Gly Phe
25 30 35

ctt gtg acc agg cat agt caa aca ata gat gac cca cag tgt cct tct 198
Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp Pro Gln Cys Pro Ser
40 45 50

ggg acc aaa att ctt tac cac ggg tac tct ttg ctc tac gtg caa ggc 246
Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu Leu Tyr Val Gln Gly
55 60 65

aat gaa cgg gcc cat ggc cag gac ttg ggc acg gcc ggc agc tgc ctg 294
Asn Glu Arg Ala His Gly Gln Asp Leu Gly Thr Ala Gly Ser Cys Leu
70 75 80 85

cgc aag ttc agc aca atg ccc ttc ctg ttc tgc aat att aac aac gtg 342
Arg Lys Phe Ser Thr Met Pro Phe Leu Phe Cys Asn Ile Asn Asn Val
90 95 100

tgc aac ttt gca tca cga aat gac tac tcg tac tgg ctg tcc acc cct 390
Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro
105 110 115

gag ccc atg ccc atg tca atg gca ccc atc acg ggg gaa aac ata aga 438
Glu Pro Met Pro Met Ser Met Ala Pro Ile Thr Gly Glu Asn Ile Arg
120 125 130

cca ttt att agt agg tgt gct gtg tgt gag gcg cct gcc atg gtg atg 486
Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Met Val Met
135 140 145

gcc gtg cac agc cag acc att cag atc cca ccg tgc ccc agc ggg tgg 534
Ala Val His Ser Gln Thr Ile Gln Ile Pro Pro Cys Pro Ser Gly Trp
150 155 160 165

tcc tcg ctg tgg atc ggc tac tct ttt gtg atg cac acc agc gct ggt 582
Ser Ser Leu Trp Ile Gly Tyr Ser Phe Val Met His Thr Ser Ala Gly
170 175 180

gca gaa ggc tct ggc caa gcc ctg gcg tcc ccc ggc tcc tgc ctg gag 630
Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro Gly Ser Cys Leu Glu
185 190 195

gag ttt aga agt gcg cca ttc atc gag tgt cac ggc cgt ggg acc tgc 678
Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His Gly Arg Gly Thr Cys
200 205 210

aat tac tac gca aac gct tac agc ttt tgg ctc gcc acc ata gag agg 726
Asn Tyr Tyr Ala Asn Ala Tyr Ser Phe Trp Leu Ala Thr Ile Glu Arg
215 220 225

agc gag atg ttc aag aag cct acg ccg tcc acc ttg aag gca ggg gag 774
Ser Glu Met Phe Lys Lys Pro Thr Pro Ser Thr Leu Lys Ala Gly Glu
230 235 240 245

ctg cgc acg cac gtc agc cgc tgc caa gtc tgt atg aga aga aca 819
Leu Arg Thr His Val Ser Arg Cys Gln Val Cys Met Arg Arg Thr
250 255 260

taatgaagcc tgactcagct accgcgggcc ctattctata gtgtcaccta aatgctagag 879

ctcgctgatc agcctcgact g 900

<210> 2
<211> 260
<212> PRT
<213> Human

<400> 2
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser
20 25 30

Val Asp His Gly Phe Leu Val Thr Arg His Ser Gln Thr Ile Asp Asp
35 40 45

Pro Gln Cys Pro Ser Gly Thr Lys Ile Leu Tyr His Gly Tyr Ser Leu

50 55 60

Leu Tyr Val Gln Gly Asn Glu Arg Ala His Gly Gln Asp Leu Gly Thr
65 70 75 80

Ala Gly Ser Cys Leu Arg Lys Phe Ser Thr Met Pro Phe Leu Phe Cys
85 90 95

Asn Ile Asn Asn Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr
100 105 110

Trp Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met Ala Pro Ile Thr
115 120 125

Gly Glu Asn Ile Arg Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala
130 135 140

Pro Ala Met Val Met Ala Val His Ser Gln Thr Ile Gln Ile Pro Pro
145 150 155 160

Cys Pro Ser Gly Trp Ser Ser Leu Trp Ile Gly Tyr Ser Phe Val Met
165 170 175

His Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro
180 185 190

Gly Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His
195 200 205

Gly Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ala Tyr Ser Phe Trp Leu
210 215 220

Ala Thr Ile Glu Arg Ser Glu Met Phe Lys Lys Pro Thr Pro Ser Thr
225 230 235 240

Leu Lys Ala Gly Glu Leu Arg Thr His Val Ser Arg Cys Gln Val Cys
245 250 255

Met Arg Arg Thr
260

<210> 3
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(813)

<400> 3
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc 54
Met Arg Ala Trp Ile
1 5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc 102

Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
10 15 20

gac tac aag gac gac gat gac aag cta gcc gtc agc atc ggc tac ctc 150
Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Val Ser Ile Gly Tyr Leu
25 30 35

ctg gtg aag cac agc cag acg gac cag gag ccc atg tgc ccg gtg ggc 198
Leu Val Lys His Ser Gln Thr Asp Gln Glu Pro Met Cys Pro Val Gly
40 45 50

atg aac aaa ctc tgg agt gga tac agc ctg ctg tac ttc gag ggc cag 246
Met Asn Lys Leu Trp Ser Gly Tyr Ser Leu Leu Tyr Phe Glu Gly Gln
55 60 65

gag aag gcg cac aac cag gac ctg ggg ctg gcg ggc tcc tgc ctg gcg 294
Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala Gly Ser Cys Leu Ala
70 75 80 85

cgg ttc agc acc atg ccc ttc ctg tac tgc aac cct ggt gat gtc tgc 342
Arg Phe Ser Thr Met Pro Phe Leu Tyr Cys Asn Pro Gly Asp Val Cys
90 95 100

tac tat gcc agc cgg aac gac aag tcc tac tgg ctc tct acc act gcg 390
Tyr Tyr Ala Ser Arg Asn Asp Lys Ser Tyr Trp Leu Ser Thr Thr Ala
105 110 115

ccg ctg ccc atg atg ccc gtg gcc gag gac gag atc aag ccc tac atc 438
Pro Leu Pro Met Met Pro Val Ala Glu Asp Glu Ile Lys Pro Tyr Ile
120 125 130

agc cgc tgt tct gtg tgt gag gcc ccg gcc atc gcc atc gcg gtc cac 486
Ser Arg Cys Ser Val Cys Glu Ala Pro Ala Ile Ala Ile Ala Val His
135 140 145

agt cag gat gtc tcc atc cca cac tgc cca gct ggg tgg cgg agt ttg 534
Ser Gln Asp Val Ser Ile Pro His Cys Pro Ala Gly Trp Arg Ser Leu
150 155 160 165

tgg atc gga tat tcc ttc ctc atg cac acg gcg gcg gga gac gaa ggc 582
Trp Ile Gly Tyr Ser Phe Leu Met His Thr Ala Ala Gly Asp Glu Gly
170 175 180

ggt ggc caa tca ctg gtg tca ccg ggc agc tgt cta gag gac ttc cgc 630
Gly Gly Gln Ser Leu Val Ser Pro Gly Ser Cys Leu Glu Asp Phe Arg
185 190 195

gcc aca cca ttc atc gaa tgc aat gga ggc cgc ggc acc tgc cac tac 678
Ala Thr Pro Phe Ile Glu Cys Asn Gly Gly Arg Gly Thr Cys His Tyr
200 205 210

tac gcc aac aag tac agc ttc tgg ctg acc acc att ccc gag cag agc 726
Tyr Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr Ile Pro Glu Gln Ser
215 220 225

ttc cag ggc tcg ccc tcc gcc gac acg ctc aag gcc ggc ctc atc cgc 774
Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu Lys Ala Gly Leu Ile Arg

230 235 240 245

aca cac atc agc cgc tgc cag gtg tgc atg aag aac ctg tgagccggcg 823
Thr His Ile Ser Arg Cys Gln Val Cys Met Lys Asn Leu
250 255

cgtgccaggg ccctattcta tagtgtcacc taaatgctag agctcgctga tcagcctcga 883

ctgtgccttc tagttgc 900

<210> 4
<211> 258
<212> PRT
<213> Human

<400> 4
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Val
20 25 30

Ser Ile Gly Tyr Leu Leu Val Lys His Ser Gln Thr Asp Gln Glu Pro
35 40 45

Met Cys Pro Val Gly Met Asn Lys Leu Trp Ser Gly Tyr Ser Leu Leu
50 55 60

Tyr Phe Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala
65 70 75 80

Gly Ser Cys Leu Ala Arg Phe Ser Thr Met Pro Phe Leu Tyr Cys Asn
85 90 95

Pro Gly Asp Val Cys Tyr Tyr Ala Ser Arg Asn Asp Lys Ser Tyr Trp
100 105 110

Leu Ser Thr Thr Ala Pro Leu Pro Met Met Pro Val Ala Glu Asp Glu
115 120 125

Ile Lys Pro Tyr Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ala Ile
130 135 140

Ala Ile Ala Val His Ser Gln Asp Val Ser Ile Pro His Cys Pro Ala
145 150 155 160

Gly Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr Ala
165 170 175

Ala Gly Asp Glu Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser Cys
180 185 190

Leu Glu Asp Phe Arg Ala Thr Pro Phe Ile Glu Cys Asn Gly Gly Arg
195 200 205

Gly Thr Cys His Tyr Tyr Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr

```
    210                 215                 220

Ile Pro Glu Gln Ser Phe Gln Gly Ser Pro Ser Ala Asp Thr Leu Lys
225                 230                 235                 240

Ala Gly Leu Ile Arg Thr His Ile Ser Arg Cys Gln Val Cys Met Lys
                245                 250                 255

Asn Leu
```

<210> 5
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(843)

<400> 5

```
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc      54
                                           Met Arg Ala Trp Ile
                                           1               5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc ccg cta gcc    102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
                10                  15                  20

gac tac aag gac gac gat gac aaa cgt gga gac agt gga tca cct gca    150
Asp Tyr Lys Asp Asp Asp Asp Lys Arg Gly Asp Ser Gly Ser Pro Ala
            25                  30                  35

acc tgg aca acg aga ggc ttt gtc ttc acc cga cac agt caa acc aca    198
Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg His Ser Gln Thr Thr
        40                  45                  50

gca att cct tca tgt cca gag ggg aca gtg cca ctc tac agt ggg ttt    246
Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro Leu Tyr Ser Gly Phe
    55                  60                  65

tct ttt ctt ttt gta caa gga aat caa cga gcc cac gga caa gac ctt    294
Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala His Gly Gln Asp Leu
70                  75                  80                  85

gga act ctt ggc agc tgc ctg cag cga ttt acc aca atg cca ttc tta    342
Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr Thr Met Pro Phe Leu
                90                  95                  100

ttc tgc aat gtc aat gat gta tgt aat ttt gca tct cga aat gat tat    390
Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr
            105                 110                 115

tca tac tgg ctg tca aca cca gct ctg atg cca atg aac atg gct ccc    438
Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro Met Asn Met Ala Pro
        120                 125                 130
```

```
att act ggc aga gcc ctt gag cct tat ata agc aga tgc act gtt tgt     486
Ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser Arg Cys Thr Val Cys
    135                 140                 145

gaa ggt cct gcg atc gcc ata gcc gtt cac agc caa acc act gac att     534
Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser Gln Thr Thr Asp Ile
150                 155                 160                 165

cct cca tgt cct cac ggc tgg att tct ctc tgg aaa gga ttt tca ttc     582
Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp Lys Gly Phe Ser Phe
                170                 175                 180

atc atg ttc aca agt gca ggt tct gag ggc gcc ggg caa gca ctg gcc     630
Ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala Gly Gln Ala Leu Ala
            185                 190                 195

tcc ccc ggc tcc tgc ctg gaa gaa ttc cga gcc agc cca ttt cta gaa     678
Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ala Ser Pro Phe Leu Glu
        200                 205                 210

tgt cat gga aga gga acg tgc aac tac tat tca aat tcc tac agt ttc     726
Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ser Asn Ser Tyr Ser Phe
    215                 220                 225

tgg ctg gct tca tta aac cca gaa aga atg ttc aga aag cct att cca     774
Trp Leu Ala Ser Leu Asn Pro Glu Arg Met Phe Arg Lys Pro Ile Pro
230                 235                 240                 245

tca act gtg aaa gct ggg gaa tta gaa aaa ata ata agt cgc tgt cag     822
Ser Thr Val Lys Ala Gly Glu Leu Glu Lys Ile Ile Ser Arg Cys Gln
                250                 255                 260

gtg tgc atg aag aaa aga cac tgagggccct attctatagt gtcacctaaa       873
Val Cys Met Lys Lys Arg His
            265

tgctagagct cgctgatcag cctcgac                                       900
```

```
<210> 6
<211> 268
<212> PRT
<213> Human

<400> 6
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
  1               5                  10                  15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Arg Gly Asp
            20                  25                  30

Ser Gly Ser Pro Ala Thr Trp Thr Thr Arg Gly Phe Val Phe Thr Arg
        35                  40                  45

His Ser Gln Thr Thr Ala Ile Pro Ser Cys Pro Glu Gly Thr Val Pro
    50                  55                  60
```

```
Leu Tyr Ser Gly Phe Ser Phe Leu Phe Val Gln Gly Asn Gln Arg Ala
65                  70                  75                  80

His Gly Gln Asp Leu Gly Thr Leu Gly Ser Cys Leu Gln Arg Phe Thr
                85                  90                  95

Thr Met Pro Phe Leu Phe Cys Asn Val Asn Asp Val Cys Asn Phe Ala
            100                 105                 110

Ser Arg Asn Asp Tyr Ser Tyr Trp Leu Ser Thr Pro Ala Leu Met Pro
        115                 120                 125

Met Asn Met Ala Pro Ile Thr Gly Arg Ala Leu Glu Pro Tyr Ile Ser
    130                 135                 140

Arg Cys Thr Val Cys Glu Gly Pro Ala Ile Ala Ile Ala Val His Ser
145                 150                 155                 160

Gln Thr Thr Asp Ile Pro Pro Cys Pro His Gly Trp Ile Ser Leu Trp
                165                 170                 175

Lys Gly Phe Ser Phe Ile Met Phe Thr Ser Ala Gly Ser Glu Gly Ala
            180                 185                 190

Gly Gln Ala Leu Ala Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ala
        195                 200                 205

Ser Pro Phe Leu Glu Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ser
    210                 215                 220

Asn Ser Tyr Ser Phe Trp Leu Ala Ser Leu Asn Pro Glu Arg Met Phe
225                 230                 235                 240

Arg Lys Pro Ile Pro Ser Thr Val Lys Ala Gly Glu Leu Glu Lys Ile
                245                 250                 255

Ile Ser Arg Cys Gln Val Cys Met Lys Lys Arg His
            260                 265


<210> 7
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(819)

<400> 7
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc      54
                                           Met Arg Ala Trp Ile
                                           1               5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc ccg cta gcc    102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
```

10 15 20

gac tac aag gac gac gat gac aag cct gga tac ctc ggt ggc ttc ctc 150
Asp Tyr Lys Asp Asp Asp Asp Lys Pro Gly Tyr Leu Gly Gly Phe Leu
25 30 35

ctg gtt ctc cac agt cag acg gac cag gag ccc acc tgc ccc ctg ggc 198
Leu Val Leu His Ser Gln Thr Asp Gln Glu Pro Thr Cys Pro Leu Gly
40 45 50

atg ccc agg ctc tgg act ggg tat agt ctg tta tac ctg gaa ggg caa 246
Met Pro Arg Leu Trp Thr Gly Tyr Ser Leu Leu Tyr Leu Glu Gly Gln
55 60 65

gag aaa gct cac aat caa gac ctt ggt ctg gca ggg tct tgc ctt ccc 294
Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala Gly Ser Cys Leu Pro
70 75 80 85

gta ttt agc acg ctg ccc ttt gcc tac tgc aac atc cac cag gtg tgc 342
Val Phe Ser Thr Leu Pro Phe Ala Tyr Cys Asn Ile His Gln Val Cys
90 95 100

cac tat gcc cag aga aac gac aga tcc tac tgg ctg gcc agc gct gcg 390
His Tyr Ala Gln Arg Asn Asp Arg Ser Tyr Trp Leu Ala Ser Ala Ala
105 110 115

ccc ctc ccc atg atg cca ctc tct gaa gag gcg atc cgc ccc tat gtc 438
Pro Leu Pro Met Met Pro Leu Ser Glu Glu Ala Ile Arg Pro Tyr Val
120 125 130

agc cgc tgt gcg gta tgc gag gcc ccg gcc cag gcg gtg gcg gtg cac 486
Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Gln Ala Val Ala Val His
135 140 145

agc cag gac cag tcc atc ccc cca tgt ccg cag acc tgg agg agc ctc 534
Ser Gln Asp Gln Ser Ile Pro Pro Cys Pro Gln Thr Trp Arg Ser Leu
150 155 160 165

tgg atc ggg tat tca ttc ctg atg cac aca gga gct ggg gac caa gga 582
Trp Ile Gly Tyr Ser Phe Leu Met His Thr Gly Ala Gly Asp Gln Gly
170 175 180

gga ggg cag gcc ctt atg tca cct ggc agc tgc ctg gaa gat ttc aga 630
Gly Gly Gln Ala Leu Met Ser Pro Gly Ser Cys Leu Glu Asp Phe Arg
185 190 195

gca gca cca ttc ctt gaa tgc cag ggc cgg cag gga act tgc cac ttt 678
Ala Ala Pro Phe Leu Glu Cys Gln Gly Arg Gln Gly Thr Cys His Phe
200 205 210

ttc gca aat aag tat agc ttc tgg ctc aca acg gtg aaa gca gac ttg 726
Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr Val Lys Ala Asp Leu
215 220 225

cag ttt tcc tct gct cca gca cca gac acc tta aaa gaa agc cag gcc 774
Gln Phe Ser Ser Ala Pro Ala Pro Asp Thr Leu Lys Glu Ser Gln Ala
230 235 240 245

```
caa cgc cag aaa atc agc cgg tgc cag gtc tgc gtg aag tat agc       819
Gln Arg Gln Lys Ile Ser Arg Cys Gln Val Cys Val Lys Tyr Ser
            250                 255                 260

taggggccct attctatagt gtcacctaaa tgctagagct cgctgatcag cctcgactgt 879

gccttctagt tgccagccat c                                           900


&lt;210&gt; 8
&lt;211&gt; 260
&lt;212&gt; PRT
&lt;213&gt; Human

&lt;400&gt; 8
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
1               5                   10                  15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Pro Gly Tyr
            20                  25                  30

Leu Gly Gly Phe Leu Leu Val Leu His Ser Gln Thr Asp Gln Glu Pro
        35                  40                  45

Thr Cys Pro Leu Gly Met Pro Arg Leu Trp Thr Gly Tyr Ser Leu Leu
    50                  55                  60

Tyr Leu Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Leu Ala
65                  70                  75                  80

Gly Ser Cys Leu Pro Val Phe Ser Thr Leu Pro Phe Ala Tyr Cys Asn
                85                  90                  95

Ile His Gln Val Cys His Tyr Ala Gln Arg Asn Asp Arg Ser Tyr Trp
            100                 105                 110

Leu Ala Ser Ala Ala Pro Leu Pro Met Met Pro Leu Ser Glu Glu Ala
        115                 120                 125

Ile Arg Pro Tyr Val Ser Arg Cys Ala Val Cys Glu Ala Pro Ala Gln
    130                 135                 140

Ala Val Ala Val His Ser Gln Asp Gln Ser Ile Pro Pro Cys Pro Gln
145                 150                 155                 160

Thr Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr Gly
                165                 170                 175

Ala Gly Asp Gln Gly Gly Gly Gln Ala Leu Met Ser Pro Gly Ser Cys
            180                 185                 190

Leu Glu Asp Phe Arg Ala Ala Pro Phe Leu Glu Cys Gln Gly Arg Gln
        195                 200                 205

Gly Thr Cys His Phe Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr
    210                 215                 220
```

```
Val Lys Ala Asp Leu Gln Phe Ser Ser Ala Pro Ala Pro Asp Thr Leu
225                 230                 235                 240

Lys Glu Ser Gln Ala Gln Arg Gln Lys Ile Ser Arg Cys Gln Val Cys
                245                 250                 255

Val Lys Tyr Ser
            260


<210> 9
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(831)

<400> 9
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc     54
                                           Met Arg Ala Trp Ile
                                           1               5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc ccg cta gct   102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
                10                  15                  20

gac tac aag gac gac gat gac aaa ggt ccc cct gga acc tcc tct gtt   150
Asp Tyr Lys Asp Asp Asp Asp Lys Gly Pro Pro Gly Thr Ser Ser Val
            25                  30                  35

gca cat gga ttt ctt att aca cgc cac agc cag aca acg gat gca cca   198
Ala His Gly Phe Leu Ile Thr Arg His Ser Gln Thr Thr Asp Ala Pro
        40                  45                  50

caa tgc cca cag gga aca ctt cag gtc tat gaa ggc ttt tct ctc ctg   246
Gln Cys Pro Gln Gly Thr Leu Gln Val Tyr Glu Gly Phe Ser Leu Leu
    55                  60                  65

tat gta caa gga aat aaa aga gcc cac ggt caa gac ttg ggg acg gct   294
Tyr Val Gln Gly Asn Lys Arg Ala His Gly Gln Asp Leu Gly Thr Ala
70                  75                  80                  85

ggc agc tgc ctt cgt cgc ttt agt acc atg cct ttc atg ttc tgc aac   342
Gly Ser Cys Leu Arg Arg Phe Ser Thr Met Pro Phe Met Phe Cys Asn
                90                  95                  100

atc aat aat gtt tgc aac ttt gct tca aga aat gac tat tct tac tgg   390
Ile Asn Asn Val Cys Asn Phe Ala Ser Arg Asn Asp Tyr Ser Tyr Trp
            105                 110                 115

ctc tct acc cca gag ccc atg cca atg agc atg caa ccc cta aag ggc   438
Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met Gln Pro Leu Lys Gly
        120                 125                 130
```

```
cag agc atc cag cca ttc att agt cga tgt gca gta tgt gaa gct cca      486
Gln Ser Ile Gln Pro Phe Ile Ser Arg Cys Ala Val Cys Glu Ala Pro
    135                 140                 145

gct gtg gtg atc gca gtt cac agt cag acg atc cag att ccc cat tgt      534
Ala Val Val Ile Ala Val His Ser Gln Thr Ile Gln Ile Pro His Cys
150                 155                 160                 165

cct cag gga tgg gat tct ctg tgg att ggt tat tcc ttc atg atg cat      582
Pro Gln Gly Trp Asp Ser Leu Trp Ile Gly Tyr Ser Phe Met Met His
                170                 175                 180

aca agt gca ggg gca gaa ggc tca ggt caa gcc cta gcc tcc cct ggt      630
Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala Leu Ala Ser Pro Gly
            185                 190                 195

tcc tgc ttg gaa gag ttt cgt tca gct ccc ttc atc gaa tgt cat ggg      678
Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe Ile Glu Cys His Gly
        200                 205                 210

agg ggt acc tgt aac tac tat gcc aac tcc tac agc ttt tgg ctg gca      726
Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ser Tyr Ser Phe Trp Leu Ala
    215                 220                 225

act gta gat gtg tca gac atg ttc agt aaa cct cag tca gaa acg ctg      774
Thr Val Asp Val Ser Asp Met Phe Ser Lys Pro Gln Ser Glu Thr Leu
230                 235                 240                 245

aaa gca gga gac ttg agg aca cga att agc cga tgt caa gtg tgc atg      822
Lys Ala Gly Asp Leu Arg Thr Arg Ile Ser Arg Cys Gln Val Cys Met
                250                 255                 260

aag agg aca taacgcggcc gctcgagcat gcatctagag ggccctattc              871
Lys Arg Thr

tatagtgtca cctaaatgct agagctcgc                                      900


<210> 10
<211> 264
<212> PRT
<213> Human

<400> 10
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
 1               5                  10                  15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Gly Pro Pro
            20                  25                  30

Gly Thr Ser Ser Val Ala His Gly Phe Leu Ile Thr Arg His Ser Gln
        35                  40                  45

Thr Thr Asp Ala Pro Gln Cys Pro Gln Gly Thr Leu Gln Val Tyr Glu
    50                  55                  60

Gly Phe Ser Leu Leu Tyr Val Gln Gly Asn Lys Arg Ala His Gly Gln
```

```
65                  70                  75                  80

Asp Leu Gly Thr Ala Gly Ser Cys Leu Arg Arg Phe Ser Thr Met Pro
                85                  90                  95

Phe Met Phe Cys Asn Ile Asn Asn Val Cys Asn Phe Ala Ser Arg Asn
            100                 105                 110

Asp Tyr Ser Tyr Trp Leu Ser Thr Pro Glu Pro Met Pro Met Ser Met
        115                 120                 125

Gln Pro Leu Lys Gly Gln Ser Ile Gln Pro Phe Ile Ser Arg Cys Ala
    130                 135                 140

Val Cys Glu Ala Pro Ala Val Val Ile Ala Val His Ser Gln Thr Ile
145                 150                 155                 160

Gln Ile Pro His Cys Pro Gln Gly Trp Asp Ser Leu Trp Ile Gly Tyr
                165                 170                 175

Ser Phe Met Met His Thr Ser Ala Gly Ala Glu Gly Ser Gly Gln Ala
            180                 185                 190

Leu Ala Ser Pro Gly Ser Cys Leu Glu Glu Phe Arg Ser Ala Pro Phe
        195                 200                 205

Ile Glu Cys His Gly Arg Gly Thr Cys Asn Tyr Tyr Ala Asn Ser Tyr
    210                 215                 220

Ser Phe Trp Leu Ala Thr Val Asp Val Ser Asp Met Phe Ser Lys Pro
225                 230                 235                 240

Gln Ser Glu Thr Leu Lys Ala Gly Asp Leu Arg Thr Arg Ile Ser Arg
                245                 250                 255

Cys Gln Val Cys Met Lys Arg Thr
            260


<210> 11
<211> 900
<212> DNA
<213> Human

<220>
<221> CDS
<222> (40)..(819)

<400> 11
ctgccgcctg cctgcctgcc actgagggtt cccagcacc atg agg gcc tgg atc      54
                                           Met Arg Ala Trp Ile
                                            1               5

ttc ttt ctc ctt tgc ctg gcc ggg agg gct ctg gca gcc cca cta gcc    102
Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu Ala Ala Pro Leu Ala
                10                  15                  20
```

```
gac tac aag gac gac gat gac aag cta gcg agc atg aga gtg ggc tac      150
Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser Met Arg Val Gly Tyr
        25                      30                      35

acg ttg gta aag cac agc cag tcg gaa cag gtg ccc ccg tgt ccc atc      198
Thr Leu Val Lys His Ser Gln Ser Glu Gln Val Pro Pro Cys Pro Ile
    40                      45                      50

ggg atg agc cag ctg tgg gtg ggg tac agc tta ctg ttt gtg gag ggg      246
Gly Met Ser Gln Leu Trp Val Gly Tyr Ser Leu Leu Phe Val Glu Gly
    55                      60                      65

caa gag aaa gcc cac aac cag gac ctg ggc ttt gct ggc tcc tgt ctg      294
Gln Glu Lys Ala His Asn Gln Asp Leu Gly Phe Ala Gly Ser Cys Leu
70                      75                      80                  85

ccc cgc ttc agc acc atg ccc ttc atc tac tgc aac atc aac gag gtg      342
Pro Arg Phe Ser Thr Met Pro Phe Ile Tyr Cys Asn Ile Asn Glu Val
                90                      95                      100

tgc cac tat gcc agg cgc aat gat aaa tct tac tgg ctc tcc act acc      390
Cys His Tyr Ala Arg Arg Asn Asp Lys Ser Tyr Trp Leu Ser Thr Thr
            105                     110                     115

gcc cct atc ccc atg atg ccc gtc agc cag acc cag att ccc cag tac      438
Ala Pro Ile Pro Met Met Pro Val Ser Gln Thr Gln Ile Pro Gln Tyr
        120                     125                     130

atc agc cgc tgc tct gtg tgt gag gca ccc tcg caa gcc att gct gtg      486
Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ser Gln Ala Ile Ala Val
    135                     140                     145

cac agc cag gac atc acc atc ccg cag tgc ccc ctg ggc tgg cgc agc      534
His Ser Gln Asp Ile Thr Ile Pro Gln Cys Pro Leu Gly Trp Arg Ser
150                     155                     160                     165

ctc tgg att ggg tac tct ttc ctc atg cac act gcc gct ggt gcc gag      582
Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr Ala Ala Gly Ala Glu
                170                     175                     180

ggt gga ggc cag tcc ctg gtc tca cct ggc tcc tgc cta gag gac ttt      630
Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser Cys Leu Glu Asp Phe
            185                     190                     195

cgg gcc act cct ttc atc gaa tgc agt ggt gcc cga ggc acc tgc cac      678
Arg Ala Thr Pro Phe Ile Glu Cys Ser Gly Ala Arg Gly Thr Cys His
        200                     205                     210

tac ttt gca aac aag tac agt ttc tgg ttg acc aca gtg gag gag agg      726
Tyr Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr Thr Val Glu Glu Arg
    215                     220                     225

cag cag ttt ggg gag ttg cct gtg tct gaa acg ctg aaa gct ggg cag      774
Gln Gln Phe Gly Glu Leu Pro Val Ser Glu Thr Leu Lys Ala Gly Gln
230                     235                     240                     245

ctc cac act cga gtc agt cgc tgc cag gtg tgt atg aaa agc ctg          819
```

Leu His Thr Arg Val Ser Arg Cys Gln Val Cys Met Lys Ser Leu
250 255 260

tagggtggca cctgccacgg gccctattct atagtgtcac ctaaatgcta gagctcgctg 879

atcagcctcg actgtgcctt c 900

<210> 12
<211> 260
<212> PRT
<213> Human

<400> 12
Met Arg Ala Trp Ile Phe Phe Leu Leu Cys Leu Ala Gly Arg Ala Leu
1 5 10 15

Ala Ala Pro Leu Ala Asp Tyr Lys Asp Asp Asp Asp Lys Leu Ala Ser
20 25 30

Met Arg Val Gly Tyr Thr Leu Val Lys His Ser Gln Ser Glu Gln Val
35 40 45

Pro Pro Cys Pro Ile Gly Met Ser Gln Leu Trp Val Gly Tyr Ser Leu
50 55 60

Leu Phe Val Glu Gly Gln Glu Lys Ala His Asn Gln Asp Leu Gly Phe
65 70 75 80

Ala Gly Ser Cys Leu Pro Arg Phe Ser Thr Met Pro Phe Ile Tyr Cys
85 90 95

Asn Ile Asn Glu Val Cys His Tyr Ala Arg Arg Asn Asp Lys Ser Tyr
100 105 110

Trp Leu Ser Thr Thr Ala Pro Ile Pro Met Met Pro Val Ser Gln Thr
115 120 125

Gln Ile Pro Gln Tyr Ile Ser Arg Cys Ser Val Cys Glu Ala Pro Ser
130 135 140

Gln Ala Ile Ala Val His Ser Gln Asp Ile Thr Ile Pro Gln Cys Pro
145 150 155 160

Leu Gly Trp Arg Ser Leu Trp Ile Gly Tyr Ser Phe Leu Met His Thr
165 170 175

Ala Ala Gly Ala Glu Gly Gly Gly Gln Ser Leu Val Ser Pro Gly Ser
180 185 190

Cys Leu Glu Asp Phe Arg Ala Thr Pro Phe Ile Glu Cys Ser Gly Ala
195 200 205

Arg Gly Thr Cys His Tyr Phe Ala Asn Lys Tyr Ser Phe Trp Leu Thr
210 215 220

Thr Val Glu Glu Arg Gln Gln Phe Gly Glu Leu Pro Val Ser Glu Thr

225 230 235 240

Leu Lys Ala Gly Gln Leu His Thr Arg Val Ser Arg Cys Gln Val Cys
245 250 255

Met Lys Ser Leu
260

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/08678

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 A61K38/39

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, PAJ, WPI Data, MEDLINE, CANCERLIT, CHEM ABS Data, EMBASE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 856 184 A (SARRAS JR MICHAEL P ET AL) 5 January 1999 (1999-01-05) column 10, line 39 -column 11, line 12; claims 1-4; figures 7,8,10 column 12, line 14 - line 32	1-4,9-16
X	PRESTAYKO ET AL: "Type IV collagen domains inhibit adhesion and migration of tumor cells and block angiogenesis" , PROCEEDINGS OF THE ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH,US,PHILADELPHIA, PA: AACR, VOL. VOL. 39, PAGE(S) 45 XP002118641 abstract	1-4,9-16

-/--

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
2 August 2000	22/08/2000

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Noë, V

Form PCT/ISA/210 (second sheet) (July 1992)

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 567 609 A (SARRAS JR MICHAEL P ET AL) 22 October 1996 (1996-10-22) abstract column 1, line 15 - line 24 column 4, line 15 - line 40 column 9, line 8 - line 19 ---	1-12
X	KEFALIDES N A ET AL: "SUPPRESSION OF TUMOR CELL GROWTH BY TYPE IV COLLGEN AND A PEPTIDE FROL THE NC1 DOMAIN OF THE ALPAH3(IV) CHAIN" MEDICINA (BUENOS AIRES), vol. 59, no. 5-2, 1999, pages 553-553, XP002144122 the whole document ---	1,13
A	HAN JING ET AL: "A cell binding domain from the alpha-3 chain of type IV collagen inhibits proliferation of melanoma cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 272, no. 33, 1997, pages 20395-20401, XP002144123 ISSN: 0021-9258 page 20400, column 2, paragraph 2; table 2 ---	13-16
A	US 5 766 591 A (BROOKS PETER ET AL) 16 June 1998 (1998-06-16) abstract column 14, line 15 - line 23; examples 7,8,10 ---	1,9,13
A	SETTY SUMAN ET AL: "Interactions of type IV collagen and its domains with human mesangial cells." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 273, no. 20, 15 May 1998 (1998-05-15), pages 12244-12249, XP002144124 ISSN: 0021-9258 page 12245, column 1, paragraph 3 ---	3,8,12, 16
P,X	WO 99 49885 A (UNIV KANSAS MEDICAL CENTER) 7 October 1999 (1999-10-07) the whole document ---	1-16
P,X	PETITCLERC E ET AL: "NEW FUNCTION FOR NON- COLLAGENOUS DOMAINS OF HUMAN COLLAGEN" JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 275, 2000, pages 8051-8061, XP002144125 abstract page 8052, column 1, paragraph 2 page 8055 -page 8057; figures 4-6,8 -----	1-4, 13-16

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Present claims 1-16 relate to an extremely large number of possible polypeptides. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the polypeptides claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to NC1 monomers of collagen type IV and their fragments.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/08678

Patent document cited in search report		Publication date	Patent family member(s)			Publication date
US 5856184	A	05-01-1999	US	5691182	A	25-11-1997
			US	5567609	A	22-10-1996
			AU	3000895	A	25-01-1996
			EP	0767676	A	16-04-1997
			WO	9600582	A	11-01-1996
US 5567609	A	22-10-1996	AU	3000895	A	25-01-1996
			EP	0767676	A	16-04-1997
			WO	9600582	A	11-01-1996
			US	5691182	A	25-11-1997
			US	5856184	A	05-01-1999
US 5766591	A	16-06-1998	US	5753230	A	19-05-1998
			AU	709645	B	02-09-1999
			AU	1995295	A	09-10-1995
			CA	2184493	A	28-09-1995
			CN	1151120	A	04-06-1997
			CZ	9602711	A	14-01-1998
			EP	0754059	A	22-01-1997
			FI	963692	A	18-09-1996
			HU	76086	A	30-06-1997
			JP	10500398	T	13-01-1998
			NO	963894	A	18-11-1996
			SK	119096	A	09-07-1997
			WO	9525543	A	28-09-1995
			ZA	9502214	A	08-02-1996
WO 9949885	A	07-10-1999	AU	3202599	A	18-10-1999